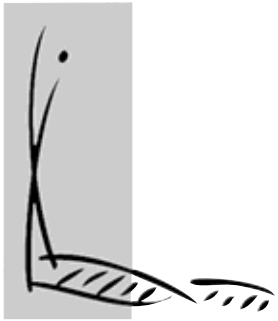
Rare Diseases and Conditions Research Activities of the National Institutes of Health

FY 2001

ANNUAL REPORT



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Annual Report on the Rare Diseases and Conditions Research Activities of the National Institutes of Health

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Executive Summary

Research on Rare Diseases Supported by NIH: FY 2001

The Orphan Drug Act of 1983 requires the Director of the National Institutes of Health (NIH) to submit a report on the rare diseases and conditions research activities supported by NIH to the Department of Health and Human Services Orphan Products Board. The rare diseases research programs sponsored by NIH Institutes and Centers (ICs) are well established and well recognized. These basic research, clinical research, and training programs continue to contribute to development and dissemination of information on prevention, etiology, diagnosis, and treatment of rare diseases and conditions.

This report presents the contributions and research advances of the fiscal year (FY) 2001 NIH extramural and intramural research programs and of the Office of Rare Diseases (ORD). Responses from the individual ICs and ORD highlight four major areas: 1) an overview of ongoing rare diseases research activities, 2) recent scientific advances in rare diseases research, 3) new or planned rare diseases research initiatives, and 4) rare disease-related activities such as workshops and symposia.

Many advances presented herein are the direct result of years of basic rare diseases research sponsored by NIH. Patients with rare diseases continue to benefit from the treatment applications realized by the emphasis NIH places on both basic and clinical research.

This report uses the definition of rare diseases as set forth in the Orphan Drug Act:

> A disease or condition with a prevalence of fewer than 200,000 people in the United States.

Prevalence refers to the number of individuals alive with the disease within a geographic parameter.

Activities undertaken in FY 2001 by ORD included cosponsoring 54 scientific workshops and symposia. The workshops continue to establish research priorities, develop program announcements, establish diagnostic and monitoring criteria, develop animal models, support of patient and tissue registries, development of research protocols and collaborative research arrangements, and disseminate workshop results.

In FY 2002, ORD is cosponsoring 58 workshops, the titles of which are included in this report. ORD is cosponsoring, with NHGRI, the Genetic and Rare Diseases Information Center. With the ICs, ORD is developing an intramural and extramural research program that will be fully operational in FY 2003 and will focus on rare diseases areas (research on groups of rare diseases) where scientific opportunities exist or research is lacking or lagging.

National Institute on Aging (NIA)

Overview

NIA conducts and supports biomedical, social, and behavioral research, training, health information dissemination, and other programs with respect to the aging process. The Institute does not focus on rare diseases per se; however, certain rare conditions/diseases are studied as they relate to the process or diseases of aging. Of particular interest are progeroid syndromes that have implications for age-related diseases, such as Werner, Bloom, and Cockayne syndromes.

Recent Scientific Advances

Werner Syndrome (WS)

WS is a rare autosomal recessive disorder characterized by genome instability and premature onset of several age-related diseases. The gene defective in WS encodes a DNA helicase (WRN) that belongs to the RecQ helicase family. Researchers have purified a multisubunit complex from human cells that contains WRN and several associated polypeptides and have found that this complex has multiple enzymatic activities. Some of the identified components could play important roles in WRN function in the prevention of premature aging.

Bloom Syndrome (BS)

BS is a rare autosomal recessive disorder characterized by short stature, sensitivity to sunlight, reduced fertility, and higher incidence of cancer. The human *BS* gene has been identified and encodes a member of the RecQ family of DNA helicases. Researchers have purified three multiprotein complexes containing the *BS* gene product and have identified most of their components. Recent studies suggest that these complexes could function as DNA-unwinding machines to repair aberrant DNA structures formed during metabolism.

Cockayne Syndrome (CS)

CS is an autosomal recessive disorder with diverse clinical symptoms that include severe mental and growth retardation, microcephaly, progressive neurological and retinal degeneration, skeletal abnormalities, and hypersensitivity to sunlight. Two genetic complementation groups, *CSA* and *CSB,* have been identified. At the cellular level, CS is characterized by a defect in transcription-coupled repair of DNA damage induced by ultraviolet (UV) light and certain forms of oxidative stress.

Intramural investigators have studied the functional significance of conserved motifs in the CSB protein to determine the biological role of *CSB* in DNA repair and transcription. These studies will increase understanding of the underlying cellular defects of CSB cells responsible for CS clinical symptoms. *CSB* mutant alleles with site-directed mutations were tested for genetic complementation of various phenotypes in both human and hamster *CSB*-null cell lines. Findings indicate that the integrity of the ATPase domain in the CSB protein is critical for cellular resistance to DNA-damaging agents, RNA synthesis, and a normal apoptotic response after treatment of cells with UV light. In contrast, a highly acidic region of the CSB protein is dispensable for DNA repair.

Current studies address the characterization of other regions of the CSB protein with genetic and biochemical assays to assess function. The role of *CSB* in repair of specific oxidative lesions and transcriptional regulation after oxidative stress with cDNA arrays is also being investigated. The CSB protein appears to be directly involved in the repair process of oxidative DNA damage removal; the underlying defects of CS (repair, transcription, or both) must be determined with a structure-function approach.

Rothmund-Thompson Syndrome (RTS)

RTS is a human genome instability disorder caused by mutation in a particular RecQ helicase. RTS shares many symptoms with WS, including predisposition to cancer and premature aging. Researchers have recently purified the

endogenous RTS gene product and are investigating its biological function.

Stickler Syndrome

This connective tissue disorder causes premature osteoarthritis, retinal detachments, and premature hearing loss. Causes include mutations in the genes encoding collagen types II and XI, but the phenotype is not linked to any of these loci in some families with Stickler syndrome. The relationship between phenotype and genetic locus in families for which the locus is known is being studied. Linkage studies are being used to search for the locus or loci causing the disorder in families for which the locus is still unknown.

Corticobasal Degeneration

Corticobasal degeneration is a rare parkinsonian disorder characterized by dementia and focal signs, in addition to the parkinsonian trait. Alien limb syndrome is a frequent part of the phenotype. Pathologically, it is characterized by neurofibrillary tangles made up of the τ protein. Recent research has shown that the τ gene is a risk-factor locus for this disease, which afflicts about 1 in 500,000 white people.

Blepharophimosis–Ptosis–Epicanthus Inversus Syndrome (BPES)

This syndrome, characterized by drooping eyelids, is associated with premature ovarian failure (POF, or early menopause) in some women. Researchers showed that the gene for a transcription factor named *FOXL2* is mutated to cause BPES. Changes in the gene cause the forms with or without POF.

Severe Achondroplasia With Developmental Delay and Acanthosis Nigricans (SADDAN)

This rare skeletal dysplasia causes severe short-limbed dwarfism with serious respiratory and neurological sequelae in infancy and young childhood. The disorder is caused by a specific missense mutation in the tyrosine kinase domain of the gene encoding fibroblast growth factor receptor type 3 (FGFR3). A mouse model for this mutation (K650M) has been developed, and genomic and proteomic strategies are being used to understand the signaling consequences of the mutation in affected organ systems.

Thanatophoric Dysplasia Type II (TDII)

TDII is among the most common of the neonatal lethal skeletal dysplasias. It is caused by a specific missense mutation in the tyrosine kinase domain of the gene encoding FGFR3. A mouse model for this mutation (K650E) has been developed, and genomic and proteomic strategies are being used to understand the signaling consequences of the mutation in affected organ systems.

Hypochondroplasia

Hypochondroplasia is among the more common and least severe of the human skeletal dysplasias. Affected individuals have short-limbed dwarfism. Learning disabilities are common with this disorder, which is caused by two specific mutations in the gene encoding FGFR3. A mouse model for one of these mutations (K650N) is under development. Genomic and proteomic strategies will be used to understand the signaling consequences of the mutation in cartilage and in the central nervous system.

Ehlers-Danlos Syndrome (EDS)

This connective tissue disorder has many varieties, most common of which are the classic and hypermobile types. Both of these forms of EDS are associated with chronic musculoskeletal pain, which may be severe and disabling. The mechanism of chronic pain in this condition and potential modes of intervention are under investigation.

Marfan Syndrome

Marfan syndrome is caused by mutations in the gene encoding fibrillin 1 (FBN1). The phenotype includes tall stature with long, thin limbs and digits; dislocated ocular lenses; and dilation and dissection of the ascending aorta. Recently developed animal models for Marfan syndrome have demonstrated several complications previously unrecognized in the human disorder. A

detailed clinical study of people with Marfan syndrome is investigating the frequency of these complications in humans.

Ectodermal Dysplasia (Anhidrotic) (EDA)

EDA is a disorder involving the lack of hair, sweat glands, and fully formed teeth (skin appendages). The gene that is mutated in humans and in the tabby mouse model produces a number of alternative protein forms. Recent studies showed that provision of one particular protein form to tabby mice restores all the appendages.

Simpson Golabi Behmel Syndrome

Simpson Golabi Behmel syndrome is an overgrowth syndrome characterized by very tall stature associated with enlarged internal organs. The gene mutated in patients, *GPC3,* produces a matrix protein around cells. Scientists have shown that disruption of the gene in mice produces comparable overgrowth by an unknown mechanism that is independent of the well-studied growth-promoting effects of insulinlike growth factors (IGFs).

Fanconi Anemia (FA)

FA is an autosomal recessive disorder characterized by diverse congenital abnormalities and a predisposition to bone marrow failure and cancer, particularly acute myelogenous leukemia. FA is comprised of eight distinct complementation groups. Researchers have purified a complex containing products of five FA genes and identified many components of this complex. They also discovered a novel biochemical activity of this complex that links this disease to DNA repair.

ATRX Syndrome

ATRX syndrome is a combination of α-thalassemia, mental retardation, and multiple developmental abnormalities. The gene defective in ATRX encodes a gene product containing an SWI2/SNF2-type DNA-dependent ATPase domain. Many proteins with this type of domain are present in multiprotein complexes, which often have ATP-dependent chromatin-remodeling activities. Researchers have purified an ATRX-containing multiprotein complex and identified most of the components of this complex. Recent studies show that the complex has multiple chromatin-modifying activities—entry points to studying its function.

von Hippel-Lindau (VHL) Syndrome

Mutation of the VHL tumor suppressor gene is responsible for VHL syndrome, a syndrome that includes hereditary renal cell carcinoma (RCC), hemangioblastomas of the cerebellum and spine, retinal angiomas, pheochromocytomas, epididymal cystadenomas, endolymphatic sac tumors, pancreatic adenomas, and islet cell tumors. Researchers are examining VHL-modulated gene expression profiles in pairs of cell lines with varying VHL status. Gene expression profiles between cells lacking functional *VHL* (parental cells) and those constitutively expressing wild-type *VHL* (*wtVHL* cells) through stable transfection are being compared. Tumor necrosis factor α (TNFα)-mediated cytotoxicity is being examined in cells with differing *VHL* status. Cells lacking functional *VHL* were found to be remarkably more resistant to TNFα-mediated killing than cells where *wtVHL* expression was restored. These results suggest that *VHL*-engendered sensitivity to the cytotoxic influence of TNFα may contribute to *VHL's* tumor suppressive function. It was also found that *VHL*-deficient RCC cells express dramatically higher levels of TNFα mRNA and protein than RCC cells expressing *VHL*. Two RNA-binding proteins exhibiting *VHL*-dependent differential association with the TNFα mRNA have been identified: HuR and AUF1.

Research Initiatives

Ongoing rare disease research projects include studies of connective tissue metabolism in Hutchinson-Gilford progeria; genetic studies of Cowden syndrome, including an investigation of the role of retinoic acid and its nuclear receptors in the disease; and several studies of the Werner syndrome gene and gene product.

Investigators in the neurobiology of aging are studying the molecular biology of prion diseases, including Creutzfeldt-Jakob disease; the neurological, cognitive, and motor performance of patients with Parkinson disease; the dementia associated with older Down syndrome patients; and the molecular and biological basis of amyotrophic lateral sclerosis (ALS).

Intramural scientists are continuing their study of premature aging disorders, including studies of DNA repair and transcription in Bloom syndrome, Cockayne syndrome, and Werner syndrome. In addition, a study is continuing on glypican-3 action in overgrowth syndromes such as Simpson Golabi Behmel syndrome.

National Institute on Alcohol Abuse and Alcoholism (NIAAA)

Overview

NIAAA conducts and supports research on the causes, consequences, and treatment of alcoholism and alcohol abuse. In addition to various liver diseases, alcoholism can cause disease in the brain, pancreas, heart, and other organs. Because the consequences of alcoholism are so diverse, knowledge gained from related research programs has can be applied to other areas of human health and disease.

Recent Scientific Advances

Alcoholic Liver Disease

Alcoholic liver disease (ALD) is a major cause of illness and mortality in the United States and around the world. It is characterized by fatty liver, hepatitis, fibrosis, and end-stage cirrhosis—which is irreversible and fatal. Developing effective treatments for ALD requires an understanding of the molecular mechanisms by which chronic ethanol exposure leads to initiation and progression of the disease. NIAAA-funded researchers have made significant progress in delineating how several molecules contribute to ALD.

Role of Endotoxin, Lipopolysaccharide (LPS), and LPS-Binding Protein (LBP)

Chronic ethanol intake is thought to increase intestinal permeability, which leads to elevated levels of endotoxin or LPS in the portal vein. Binding of LPS to Kupffer cells (liver resident macrophage) triggers a cascade of events leading to increased tumor necrosis factor α (TNFα) production. Increasing evidence suggests that cellular responses to LPS are enhanced significantly by LBP. NIAAA-funded researchers have used LBP knockout and wild-type mice to understand the significance of LBP in ALD. In wild-type mice, chronic ethanol exposure increased TNFα expression and caused steatosis, mild inflammation, and focal necrosis. However, these changes were not significant in LBP knockout mice (mice in which the *LBP* gene has been rendered nonfunctional), confirming the role of LBP in alcohol-induced liver injury via Kupffer cell activation and TNFα production.

Role of LPS Receptors—CD14 and Toll-Like Receptors

CD14 receptors are required for LPS to bind to Kupffer cells and initiate the inflammatory cascade. Toll-like receptors (TLR4) are required to transmit the signals further into the cell. NIAAA researchers used CD14 knockout and wild-type mice to evaluate the relative significance of these receptors in alcohol-induced liver injury. In wild-type mice, exposure to ethanol elevated serum alanine aminotransferase (a marker of liver injury) levels induced severe liver injury and increased the expression of TNFα and NF-κB (a nuclear transcription factor). In contrast, these effects were not significant in CD14 knockout mice. Similar results were found in mice with TLR4 mutations. These results suggest that both CD14 and TLR4 are necessary for LPS to initiate the cascade of events leading to alcohol-induced liver injury.

Role of Intercellular Adhesion Molecules-1 (ICAM-1)

ICAM-1 is an adhesion molecule that binds inflammatory leukocytes with sinusoidal epithelium. This binding is followed by migration of inflammatory cells into hepatic parenchyma, where they can damage hepatocytes by releasing free radicals and proteases. Researchers have used ICAM-1 knockout mice to assess the role of ICAM-1 in ALD. In wild-type mice, ethanol caused severe liver injury, which was associated with increased infiltration of leukocytes and increased expression of TNFα mRNA. However, these changes were not significant when ICAM-1 knockout mice were exposed to ethanol, suggesting the involvement of ICAM-1 in ALD.

Role of Oxidant Stress

Hepatic fibrosis results when activated hepatic stellate cells (HSCs) produce excess extracellular matrix proteins, especially collagen. If left untreated, fibrosis can progress to cirrhosis, which is fatal. NIAAA-funded researchers have discovered that reactive oxygen species (free radicals) derived from an ethanol-metabolizing enzyme known as cytochrome P450 2E1 (CYP2E1) can cause HSCs to produce increased amounts of collagen. When HSCs were incubated with liver cells that had been injected with CYP2E1, they proliferated and produced greater amounts of collagen type I protein, smooth muscle actin (a marker of HSC activation), and free radicals such as H_20_2 and lipid peroxidation products. Collagen production was further increased when cells were incubated with other free radical-producing agents such as arachidonic acid. The production of collagen and HSC proliferation were prevented by antioxidants such as vitamin E and catalase and by inhibiting the activity of CYP2E1. These results suggest that liver cells injected with CYP2E1 release free radicals, which can activate and proliferate HSC, causing increased collagen production, which may lead to fibrosis. The results may shape developing strategies to prevent hepatic fibrosis via antioxidant therapies.

Alcoholic Pancreatitis

Chronic alcohol consumption is associated with up to 70% of pancreatitis cases in the United States, affecting roughly 100,000 people annually. Progression of acute and chronic pancreatitis may lead to diabetes and even cancer. NIAAA-funded researchers have made significant progress in understanding the underlying mechanisms by which alcohol leads to the development of pancreatitis.

Role of Dietary Fat and Associated Oxidant Stress

Rats fed a liquid alcohol diet (up to 40% calories) containing unsaturated fat for 8 weeks exhibited elevated levels of serum pancreatic enzymes and histopathological features of steatosis, inflammation, necrosis, and fibrosis in the pancreas. These changes were associated with increased levels of carbon-centered radical adducts and 4-hydroxynonenal, an index of lipid peroxidation. Administration of alcohol with saturated fat attenuated the severity of pancreatic injury and blunted radical adduct formation. These results suggest that dietary fat type plays an important role in the pathogenesis of alcoholic pancreatitis and that this effect is mediated through increased oxidant stress.

Role of Ethanol Metabolites

Pancreatic acinar cells metabolize ethanol primarily by a nonoxidative pathway in which fatty acid ethyl ester synthase catalyzes the conversion of ethanol to fatty acid ethyl esters (FAEEs). The pancreas can also metabolize ethanol via an oxidative pathway in which alcohol dehydrogenase catalyzes the conversion of ethanol to acetaldehyde. Researchers compared the effects of FAEEs and acetaldehyde on NF-κB activation in isolated pancreatic acinar cells. This transcription factor has been implicated in the process of pancreatic inflammation via increased transcription of pro-inflammatory cytokines. Although FAEEs induced NF-κB activation, acetaldehyde inhibited the activation. Thus, ethanol may regulate NF-κB positively or negatively, depending on the predominant metabolic pathway effect. These regulatory mechanisms may play a role in the induction of pancreatitis.

Xeroderma Pigmentosum (XP)

Individuals with the genetic disease xeroderma pigmentosum (XP) lack the capacity to carry out a specific type of DNA repair reaction called nucleotide excision repair (NER). Some people with XP develop a progressive neurological degeneration believed to result from the accumulation of an endogenous DNA lesion that goes unrepaired due to NER function loss. NIAAA researchers previously identified a class of oxidative DNA lesions called cyclopurines that are attractive candidates for the endogenous DNA lesions that cause neurodegeneration in XP patients. More recently, researchers have developed a very sensitive assay to detect the presence of these lesions in tissue samples, allowing scientists to determine whether cyclopurines accumulate in the brains of XP patients. This assay also will be useful in testing different

compounds for the ability to prevent formation of this lesion. Such compounds ultimately may be useful in treating neurodegeneration in XP patients.

Fetal Alcohol Syndrome (FAS)

Prenatal exposure to alcohol can produce a spectrum of problems, including postnatal growth retardation, neurological abnormalities, developmental delays, behavioral dysfunction, intellectual impairment, and skull or brain malformations. Collectively, these abnormalities are referred to as *fetal alcohol syndrome* (FAS). Research continues on characterizing the neurodevelopmental dysfunction of children with FAS from neurodevelopmental problems attributable to other causes. NIAAA-supported researchers recently reported the results of a study aimed at characterizing general cognitive functioning in a group of South African children with FAS—the first description of cognitive-motor functioning in a group of reliably diagnosed children with FAS in a non-Western community. The findings showed that the pattern of cognitive-motor deficits in this group of children was, by and large, commensurate with that reported among FAS children in Western countries, strongly suggesting that neurobehavioral deficits resulting from prenatal alcohol exposure can be generalized across ethnic and cultural boundaries.

Numerous ongoing NIAAA studies seek to identify effective methods for preventing FAS. Many of these studies involve various forms of motivational enhancement delivered by health professionals to encourage pregnant women, especially those at high risk, to refrain from drinking alcohol. These studies target women from various socioeconomic and ethnic backgrounds. Another new FAS prevention study examines an environmental intervention strategy, focusing on servers of alcohol at bars and restaurants, and includes the dissemination of FAS information to both female and male customers.

Other NIAAA-supported investigators are examining four types of "naturally occurring" interventions that could potentially prevent FAS and related problems: alcohol tax and availability policies, point-of-sale warning signs about the hazards of drinking, physician advice regarding drinking during pregnancy, and mandated warning labels on containers of alcoholic beverages.

NIAAA continues to fund a large community-based trial of comprehensive interventions to prevent FAS among four Plains Indian tribes, with two other tribal groups serving as controls. Interventions being tested include universal educational strategies to disseminate information and change community norms, selective interventions for women at elevated risk (identified through clinic-based screening programs), and indicated interventions for high-risk women in their childbearing years (e.g., treatment programs for women who are alcohol dependent).

Research Initiatives

Alcoholic Hepatitis: Cellular and Molecular Mechanisms

Developed in collaboration with NIDDK, this program announcement seeks grant applications to study the underlying cellular, molecular, and biochemical mechanisms by which chronic alcohol ingestion leads to the initiation and development of alcoholic hepatitis.

S-Adenosylmethionine (SAMe) and Liver Disease

Developed in collaboration with the Office of Dietary Supplements (ODS) and NCCAM, this request for applications invites proposals for innovative basic and preclinical research to understand how SAMe may be effective in the treatment of liver diseases caused by alcohol, hepatitis C virus (HCV), and other factors.

National Institute of Allergy and Infectious Diseases (NIAID)

Overview

NIAID supports research activities on rare diseases that are classified into four areas: infectious diseases, primary immunodeficiency diseases, autoimmune diseases, and other immune system-mediated conditions. Infectious diseases can be caused by viruses, bacteria, fungi, and other parasites. Primary immunodeficiency diseases are hereditary disorders caused by intrinsic defects in the cells of the immune system and are characterized by unusual susceptibility to infection. In autoimmune diseases, the immune system mistakenly attacks and damages the body's own cells and tissues. Other immune system-mediated conditions include allergies, which are inappropriate or exaggerated reactions of the immune system, and chronic inflammatory conditions. NIAID research on rare diseases is aimed at delineating the mechanisms of disease pathogenesis and developing new and more effective strategies for the diagnosis, treatment, and prevention of these diseases.

Recent Scientific Advances

Rare Infectious Diseases

Amebiasis

Whether immunity to *Entamoeba histolytica*, the etiologic agent of amebiasis, can be acquired through infection is unknown. NIAID-supported researchers studying antibodies against the amebic GalNAx lectin, which mediates adherence to and lysis of human colonic cells in amebiasis, showed that children with mucosal anti-lectin antibodies were less likely to be infected with *E. histolytica* than children without mucosal anti-lectin antibodies. Likewise, children with stool anti-lectin antibodies were less likely than children without stool anti-lectin antibodies to develop new *E. hystolytica* infections. This study demonstrates that immunity can be acquired and that vaccine development for amebiasis is a possibility.

Aspergillosis

Aspergillus is one of the most common fungal pathogens affecting immunocompromised patients. NIAID intramural investigators have been dissecting the genes involved in *A. fumigatus* spore color synthesis and determining their role in virulence. Their work has demonstrated that the spore pigment synthesis pathway plays an important role in pathogenesis. The researchers recently identified one hyphae-specific gene that is clustered with six previously isolated spore pigment genes, and they are characterizing the gene and its function in the growth of hyphae (filament consisting of multiple fungal cells).

Chickenpox

Chickenpox is caused by the varicella-zoster virus (VSV). NIAID intramural scientists have developed and characterized a series of *VSV* mutants lacking individual *VZV* genes to determine genes that are important for establishing latent infection in the nervous system. Information on these mutants may assist development of safer vaccines to protect against chickenpox.

Cholera

Studies under various growth conditions have shown that *Vibrio cholerae*, the causative agent of cholera, is constantly adapting to changes in its environment. NIAID-supported investigators have now identified a gene family that allows *V. cholerae* bacteria to join together to form biofilms and, in doing so, produce an extra layer of sugars that protects the bacteria from environmental stresses and makes them more resistant to disinfection by chlorine. When conditions become more favorable, other genes allow reversion back to the free-living form. This demonstrates a mechanism that the organism can use to persist in harsh conditions.

Another scientific advance involves a new cholera vaccine that has shown promise in a NIAID-sponsored phase I/II trial. A live attenuated cholera vaccine produced by Avant Immuno

therapeutics, Inc., proved to be safe and efficacious—100% effective in protecting against moderate and severe diarrhea and 93% effective against diarrhea in general. The vaccine was well tolerated, although the rate of headaches and abdominal cramps increased after vaccination.

Cryptococcosis

Cryptococcosis, infection by the fungus *Cryptococcus neoformans*, occurs primarily in immunocompromised individuals, such as those with AIDS or undergoing immunosuppressive therapy. A class of proteins known as mannoproteins (MPs) can stimulate T cells, immune cells that are critical for defense against cryptococcosis. In a series of experiments to isolate and sequence the cryptococcal component that stimulates T cells, NIAID-supported scientists identified one of these MPs—MP98. Early tests of MP98 as a potential vaccine in mice have shown some protection against infection with *C. neoformans*.

NIAID intramural scientists are trying to determine the factors that enable *C. neoformans* to cause disease in humans while other genetically related fungi remain nonpathogenic. They have identified four capsule genes that are essential for virulence and are studying their regulation and expression. This work may lead to new drug targets.

Cryptosporidiosis

Cryptosporidiosis is a common cause of diarrheal disease. To date, only two types *of Cryptosporidium parvum* were thought to cause disease in immunocompetent people, although a wider range of *Cryptosporidium* species were detected in patients with AIDS. Using new molecular tools, NIAID-supported researchers determined the genetic characteristics of *Cryptosporidium* parasites collected from Peruvian children in a study of diarrheal disease. The results revealed that in a setting where cryptosporidiosis is a common endemic childhood infection, novel species and types can infect human immunodeficiency virus (HIV)-negative children.

Cytomegalovirus (CMV)

Congenital infection. Congenital CMV infections are the most common nongenetic cause of hearing loss in infants. Approximately 1% of all infants born in the United States have congenital CMV infection. Clinical evaluation of the safety and efficacy of intravenous ganciclovir treatment of symptomatic central nervous system congenital CMV infections demonstrated that newborns who received treatment showed improved hearing function and less hearing deterioration at 6 months. Based on these studies, intravenous ganciclovir has been established as standard care for symptomatic congenital CMV infections.

Retinitis. In a group of patients with inactive CMV retinitis, NIAID intramural investigators demonstrated that it is safe to discontinue anti-CMV maintenance therapy after highly active antiretroviral therapy (HAART) increases $CD4^+$ T-cell counts.

Dengue

NIAID-supported researchers have successfully demonstrated two promoters that can drive the expression of foreign DNA in the genome of *Aedes aegypti*, the mosquito carrier of yellow fever and dengue. These promoters are specific to the mosquito midgut region and are expressed at the highest levels within 24 hours of a blood meal. The promoters will enable scientists to insert genes that will hinder pathogen development in the mosquito and thus interrupt the transmission of the parasites to humans.

NIAID intramural investigators demonstrated in a clinical trial that a live attenuated dengue type 4 vaccine candidate was safe and immunogenic. The vaccine candidate was well tolerated, did not cause systemic illness in any of the volunteers, and was not transmitted to mosquitoes. This was the first live attenuated *Flavivirus* vaccine candidate to be evaluated in humans.

Escherichia coli O157:H7

Most strains of *Escherichia coli* are members of the normal human gut flora, but a strain known as *E. coli* O157:H7 is a life-threatening intestinal pathogen. Infection can lead to bloody diarrhea

and hemolytic uremic syndrome, which can be especially problematic in young children, the elderly, and people with decreased immune function. In an effort to understand the similarities and differences between pathogenic and nonpathogenic strains of *E. coli,* NIAID-supported researchers sequenced the genome of *E. coli* O157:H7 and compared it with the sequence of the nonpathogenic *E. coli* K12. The researchers determined that the genome of *E. coli* O157:H7 is about 30% larger than that of the nonpathogenic strain and includes genes that code for toxins and other virulence factors.

Giardiasis

Giardiasis is intestinal infection with the protozoan parasite *Giardia,* resulting in diarrhea. NIAID intramural investigators have been studying the surface antigen variation of *Giardia,* which allows the parasite to survive and multiply in the intestine. By destroying several conserved features common to variable surface proteins, they demonstrated that the protein's conserved lipidlike tail and a part that binds zinc are essential to making and moving the protein to the parasite's surface. These could be new therapeutic targets.

Haemophilus influenzae

Haemophilus influenzae can invade and pass through the epithelium to reside within the mucosa. NIAID-supported researchers found that *H. influenzae*-induced perturbation of the epithelial barrier was correlated with disruption of structures known as tight junctions and that the host's nitric oxide response may be harnessed by *H. influenzae* to compromise epithelial integrity. Researchers have successfully modulated this cellular response to preserve tight junction complexes during infection and are currently determining the effect this has on *H. influenzae* invasion.

Another scientific advance pertains to the potential of *H. influenzae* high-molecular-weight (HMW) adhesion proteins as a vaccine candidate. NIAID-supported researchers recently demonstrated that naturally produced human antibodies directed against these *H. influenzae* proteins are capable of binding to and killing the bacteria in a standard laboratory assay. Immunization of chinchillas with several prototype *Haemophilus* HMW proteins resulted in the production of antibodies that killed not only the original strain from which each protein was purified but several unrelated strains as well.

Hepatitis E Virus (HEV)

NIAID intramural scientists have characterized a new HEV strain recovered from infected swine. Epidemiological studies of swine handlers have shown that they have excess HEV antibodies, suggesting that the virus may be spread from animals to humans. NIAID investigators also successfully transmitted an HEV-like agent from rats captured in Los Angeles to laboratory rats of the same species. Studies to determine whether rat HEV is linked to human infection are in progress.

Herpes Simplex Virus (HSV): Neonatal Infection

NIAID's Collaborative Antiviral Study Group (CASG) investigators established the safety and effectiveness of a new dose of the antiviral drug acyclovir for treatment of neonatal herpesvirus infections. Despite treatment, children with neonatal herpesvirus infection often die or suffer from morbidity, for example, blindness. To address this, researchers conducted a study to determine whether a higher dose of acyclovir could lead to improvement. The study demonstrated that a high dose of acyclovir significantly reduced mortality in newborns with disseminated HSV infection, the most severe form of HSV infection. In addition, the study determined that destruction of immune cells could be a side effect of acyclovir. This provides important new information for physicians who are managing patients on acyclovir.

Histoplasmosis

Histoplasma capsulatum is found as mold in nature, but it lives as yeast inside immune cells known as macrophages in the human host. *H. capsulatum* produces calcium-binding protein, which has been identified as a virulence factor. NIAID-funded researchers made a mutant strain of *H. capsulatum* by disrupting the gene that

codes for calcium-binding protein, so the protein could no longer be made. The mutant strain could not destroy macrophages grown in laboratory culture. Repair of the gene generated a strain that could destroy macrophages as well as the original strain from which the mutant was made. This knowledge may lead to new targets for drugs that would keep the fungus from destroying macrophages but be less harmful to infected humans.

Kaposi Sarcoma (KS)

NIAID intramural investigators discovered that ORF74, a receptor encoded by the KS-associated herpesvirus HHV-8, can bind human chemoattractants and activate NF-κB, which induces the expression of various growth factor genes. This work unifies the infectious and growth factor theories of KS pathogenesis and suggests that this receptor may be a good therapeutic target.

Kawasaki Disease

NIAID intramural scientists attempted to transmit a putative infectious agent from plasma samples of children with Kawasaki disease to chimpanzees. The chimpanzees demonstrated extreme hypersensitivity to the inoculum. Initial characterization of the reactive substance in the plasma of Kawasaki patients revealed that it was a heat-stable macromolecule. The molecule is being further characterized.

Leishmaniasis

NIAID-supported investigators have shown that the level of a compound called tetrahydrobiopterin (BH_4) controls *Leishmania* development. Genetically manipulated parasites lacking the enzyme pteridine reductase contain low levels of BH_4. These mutant parasites cause more severe disease, as measured by greater numbers of parasites found in the body and larger skin lesions resulting from infection. The investigators concluded that, by means of pteridine metabolism, *Leishmania* may have evolved the ability to limit its ability to cause disease and thus enhance persistence and transmission.

NIAID intramural scientists developed a *Leishmania* vaccine consisting of salivary proteins of the sand fly vector. This vaccine provided powerful protection against *Leishmania* infection in mice and demonstrated that salivary proteins or their cDNA are viable vaccine candidates against leishmaniasis.

NIAID intramural scientists also characterized the genes encoding several different surface membrane enzymes of *Leishmania,* which are unique to these parasites and critical for their survival. As such, they provide logical targets for design and development of new chemotherapeutic agents, diagnostic tools, and potential vaccines.

Lyme Disease

NIAID-supported researchers compared the reliability of blood versus urine laboratory tests for Lyme disease. The study showed that blood tests with a commercially available IgG Western blot kit gave 100% concordant and highly reproducible results. In contrast, urine tests gave contradictory results and a high percentage of false-positive results. These findings suggest that the Lyme urine antigen test is unreliable and should not be used for the laboratory diagnosis of Lyme disease.

Another group of NIAID-supported researchers showed that immunization with decorin-binding protein A (DbpA) of *Borrelia burgdorferi* failed to protect mice from infection transmitted through ticks, even though previous studies have shown that mice were protected on intradermal challenge with *B. burgdorferi* cultured in vitro. This result is consistent with the fact that DbpA could not be detected on *B. burgdorferi* grown in ticks and suggests that DbpA may not be appropriate as a vaccine against Lyme disease.

Little is known about the interaction of *B. burgdorferi* with the host immune system in chronic infection. NIAID-funded researchers examined the relationship between the types of antibodies produced and the bacterial load in dexamethasone-treated (immunosuppressed) monkeys. The results showed that, although IgG antibody levels were substantially decreased in dexamethasone-treated monkeys, IgM antibody levels were in excess of those detected in immu

nocompetent monkeys. Despite high levels of IgM antibodies against *B. burgdorferi,* bacterial loads were much higher in these animals. These findings indicate that IgG antibodies are more effective than IgM antibodies in reducing the number of *B. burgdorferi* in infected animals.

Because ticks that transmit Lyme disease are often coinfected with the pathogen that causes human granulocytic ehrlichiosis, NIAID-supported scientists examined the influence of infection with both pathogens on the course of Lyme-associated arthritis and granulocytic ehrlichiosis in mice. Dual infection resulted in more severe arthritis than that resulting from infection with *B. burgdorferi* alone and resulted in changes in the levels of various cytokines that regulate host immune responses. During dual infection, the expression of interferon-γ receptors on macrophages was reduced, implying a decrease in the ability of macrophages to destroy bacterial pathogens.

Analysis of patients' responses to a quality-of-life questionnaire from two NIAID-funded clinical trials revealed that antibiotic therapy (treatment with ceftriaxone and doxycycline) is not more effective than placebo in improving chronic Lyme disease symptoms. Study volunteers who received standard antibiotic treatment for Lyme disease suffered from persisting physical and cognitive problems related to their illness. In addition, investigators found no evidence of the Lyme disease bacterium in blood or spinal fluid from patients with chronic symptoms. These findings, coupled with the knowledge of treatment of other chronic infectious diseases caused by persistent bacteria, suggest that a longer course of antibiotic therapy or different antibiotic combinations would probably not improve chronic symptoms.

Lymphogranuloma Venereum (LGV)

Chlamydia trachomatis isolates are separated into variants based on the disease they cause. NIAID intramural investigators analyzed the genomes of a variant that causes sexually transmitted disease (STD) and a variant that causes LGV. They discovered that the STD variant genome had a gene family with homology to that of the clostridial toxin B, which was not present in the LGV variant genome. Additional studies demonstrated that the STD variant produces a chlamydial toxin that is delivered to host cells very early during infection. Future work will focus on understanding how the toxin functions, how it affects the biology of chlamydial infection, and whether it is a target of the host's immune response.

Marburg Virus

NIAID intramural scientists have determined the three-dimensional structure of a membrane protein of the Marburg virus—matrix protein vp40. This protein represents 30% of the viral particle and is essential for viral structure and assembly. This work will lead to greater understanding of viral infection, replication, and pathogenicity.

Meningococcal Infection

Searching the genome databases of *Neisseria meningitidis* and *N. gonorrhoeae,* NIAID-supported investigators identified and characterized three new bacterial outer membrane proteins. One of the surface proteins they identified was present on all strains of *N. meningitidis* examined. Future efforts will attempt to determine their usefulness as vaccine candidates.

Microsporidiosis

NIAID intramural scientists have identified several parasite proteins that are important for survival in the host, including two spore wall proteins in the important species *Encephalitozoon intestinalis.* Understanding how the spore wall forms is important because the spore wall allows the parasite to survive outside the host; interrupting the process may lead to treatment and prevention of infections.

Pertussis

Pertussis, also known as whooping cough, is rarely considered or diagnosed in older children or adults. NIAID recently completed a prospective multisite trial on the safety and efficacy of an acellular pertussis vaccine designed for use in older individuals. The acellular vaccine was shown to be efficacious and safe. Extensive experience in children suggests that an acellular

pertussis vaccine given to adolescents and adults in the form of a DTaP booster would be effective in reducing the disease burden in this population.

Plague

NIAID intramural scientists and colleagues are studying the plague organism *(Yersinia pestis)* and its vector, including developing the ability to infect mouse and rat models with fleas carrying plague. The mouse model will be used to evaluate a new recombinant vaccine, developed by the U.S. Army Medical Research Institute of Infectious Disease, for its ability to protect mice against flea-borne transmission of *Y. pestis*.

These studies also found that a single gene change in a relatively benign recent ancestor of the plague bacterium plays a key role in the evolution of the deadly disease. By acquiring this gene, the bacterium gradually changes from a germ that causes a mild human stomach illness acquired via contaminated food or water to the flea-borne agent of the Black Death, which killed one-fourth of Europe's population in the 14th century. This research contributes to understanding the forces behind the emergence of plague. The gene allowed the bacteria to be transmitted through the bite of an insect—in this case, the flea—an adaptation that distinguishes *Y. pestis* from all closely related, more benign gut bacteria.

Pneumocystis Pneumonia

Pneumocystis pneumonia, caused by *Pneumocystis carinii,* is the most frequent opportunistic infection associated with AIDS and can be fatal if untreated. Trimethoprim-sulfamethoxazole (TMP/SMX or cotrimoxazole) is the primary antibiotic for prophylaxis and treatment in HIV-infected individuals. NIAID-supported investigators recently demonstrated that mutations in the gene target of TMP/SMX occur in *Pneumocystis* organisms from AIDS patients. The investigators noted a positive correlation between the duration of treatment and the risk of mutations resulting in treatment failure. These findings suggest a trend toward resistance to TMP/SMX.

Poliomyelitis

Combining the strengths of two approaches to drug design (i.e., structure-based design and combinatorial chemistry), NIAID-supported researchers developed the novel structurally biased combinatorial approach to drug design. With this approach, they have designed several new lead compounds that are active against poliomyelitis virus or poliovirus.

NIAID intramural scientists have been conducting studies to understand the molecular mechanisms responsible for poliovirus replication. They recently delineated the requirements for assembly of poliovirus replication complexes and negative-strand RNA synthesis. The findings will enable rational design of antiviral chemotherapeutic agents that inhibit specific steps in viral RNA replication, as well as development of viral vaccines.

Rabies

NIAID intramural researchers are developing a DNA vaccine for rabies and have demonstrated that one-time intramuscular vaccination with rabies DNA can protect nonhuman primates against rabies virus challenge more than 1 year after vaccination. This work suggests that it is possible to produce a highly efficacious and inexpensive rabies DNA vaccine to replace the dangerous nervous tissue vaccines and the expensive tissue culture vaccines.

Spongiform Encephalopathy

The infectious agent associated with transmissible spongiform encephalopathy (TSE) is the abnormal prion protein PrP-res, which can cause normal PrP to convert to PrP-res. NIAID intramural investigators identified regions of PrP-res that are folded differently as a function of the TSE strain. They also determined that PrP-res and normal PrP have to be colocalized in the same membrane for efficient conversion.

NIAID researchers studying the transmission of spongiform encephalopathy across species have uncovered evidence of a molecular barrier that limits susceptibility of humans, cattle, and sheep to chronic wasting disease. They revealed that species that were once thought to be resistant to

certain forms of transmissible spongiform encephalopathy can serve as lifelong carriers of the infection without ever becoming sick.

Streptococcus, Group A

Streptococcus pyogenes, also known as group A streptococcus (GAS), is responsible for a wide variety of diseases, including strep throat, scarlet fever, skin infections, acute rheumatic fever, acute glomerulonephritis (infection of the kidney), toxic shock syndrome, and necrotizing fasciitis (also known as flesh-eating disease). By determining the genomic sequence of *S. pyogenes,* NIAID-supported scientists found more than 40 virulence genes that contribute to GAS's ability to cause disease. This information is expected to lead to new treatments and vaccine candidates for GAS infections.

Another group of NIAID-supported investigators reported that lysin, an enzyme produced by bacteriophage (virus that infects bacteria), can kill GAS on contact. The investigators reported that lysin prevented and eliminated GAS colonization of the upper respiratory tract in mice. Use of lysin could be explored as a novel approach to controlling streptococcal invasive diseases.

NIAID intramural investigators sequenced and characterized the genome of GAS strain MGAS8232 (serotype M18) isolated from a patient with acute rheumatic fever and compared it to the genome of strain SF370 (serotype M1 strain). The investigators found that strain MGAS8232 had genes encoding many additional secreted proteins, including streptococcal pyrogenic exotoxin A (scarlet fever toxin) and two uncharacterized proteins resembling pyrogenic exotoxins (fever-causing toxins). This work provides a foundation for accelerated research into the pathogenesis of acute rheumatic fever.

Another group of NIAID intramural scientists used genome sequencing and other analytical techniques to identify 11 molecules of GAS that can be studied for their suitability as human vaccine components. All GAS strains examined from worldwide sources produced these molecules, and they were produced when the bacterium infected humans.

Streptococcus, Group B

Group B streptococcus (GBS) causes significant morbidity and mortality in neonates, pregnant women, and immunocompromised adults. Streptococcal C5a peptidase (SCPB), a surface protein produced by all GBS serotypes, could serve as a potential vaccine candidate to protect against a broad range of GBS. NIAID-supported investigators demonstrated that exposure of GBS to antibodies against SCPB resulted in rapid killing of GBS by macrophages (cells of the immune system that engulf foreign microorganisms). Studies are testing whether vaccination with SCPB can protect mice against GBS.

Streptococcus pneumoniae

Streptococcus pneumoniae is the leading cause of meningitis. NIAID-supported scientists determined that *S. pneumoniae* kills brain cells by triggering programmed cell death. These scientists have also determined that *S. pneumoniae* enters the bloodstream through the pathway by which immunoglobulins are secreted onto the cell surface. The *S. pneumoniae* protein that binds to the IgA receptor, known as choline binding protein A, could be a candidate for vaccine. Finally, this group of scientists discovered that mutations in the *S. pneumoniae* death pathway result in bacteria that can no longer be killed, even by the last-line drug vancomycin. These mutations exist in up to 20% of *S. pneumoniae* circulating in the general community, and 2–4% of these infections will not respond to antibiotics.

Another group of NIAID-supported scientists determined the genome sequence of a virulent isolate of *S. pneumoniae* (serotype 4). Comparison of the DNA sequence of the virulent isolate with the sequence of other strains revealed that approximately 10% of the genes in this virulent strain are missing from the other nonvirulent strains, and these genes could contribute to differences in virulence. Sequence analysis identified *S. pneumoniae* genes encoding enzymes that may be important for the synthesis of bacterial capsule and for weakening biological mem

branes. The analysis also predicted 69 surface proteins that may serve as vaccine candidates.

NIAID-supported trials in Navajo/Apache children demonstrated that Prevnar (Wyeth), the newly licensed pneumococcal conjugate vaccine, protects against invasive pneumococcal disease. Vaccinated children were also less likely to be carriers of the seven serotypes of *S. pneumoniae* targeted by the vaccine.

Recently, NIAID-supported scientists found that two surface proteins, CppA and PhpA, are commonly present in almost all strains of *S. pneumoniae* isolated from patients. The investigators determined that purified CppA and PhpA can protect mice from respiratory infection and death after intravenous exposure to *S. pneumoniae.* A different group of NIAID-supported scientists identified PspA, another surface protein expressed by all strains of *S. pneumoniae.* They conducted a phase I clinical trial of PspA, and showed the protein elicit an immune response. When administered to mice, the human antibodies to PspA protected mice from pneumococcal infection. These proteins may provide alternatives to current vaccines.

Tetanus

NIAID-supported researchers are developing an oral vaccine based on a live, weakened strain of *Salmonella typhi* to prevent tetanus. This strain of *S. typhi* has been genetically engineered to successfully secrete proteins of *Clostridium tetani,* the etiologic agent of tetanus. In a phase I clinical trial, researchers demonstrated that an oral dose of the engineered *S. typhi*-based tetanus vaccine can elicit the production of protective antibodies.

Toxoplasmosis

Toxoplasma gondii, which causes toxoplasmosis and infectious retinitis, is classified into types I, II, and III. Previous studies showed that parasites isolated from patients with congenital infection or immunocompromised patients with symptomatic infection were largely type II. NIAID-funded investigators have shown that the more virulent type I is found in immunocompetent patients with severe toxoplasmic retinochoroiditis. Identification of genetic differences among the different strains may yield insight into the basic mechanisms of disease and new treatment strategies for immunocompetent adults.

Another group of NIAID-supported investigators studied changes in host cell metabolism and gene expression that occur when *T. gondii* invades and develops within human cells. The study showed increased expression of host genes associated with the immune response. These effects were also seen with soluble factors secreted by extracellular parasites, indicating that the effects are not dependent on invasion or cell-to-cell contact. The parasite also affected host genes associated with sugar and cholesterol metabolism. These results suggest that the stress of parasitism leads to an anaerobic environment, which induces increased expression of sugar-metabolizing genes. The changes in gene expression associated with cholesterol probably result from the parasite scavenging cholesterol from its host.

West Nile Virus (WNV)

The most serious manifestation of WNV in humans is fatal encephalitis (inflammation of the brain). NIAID-supported researchers have developed a hamster model of WNV encephalitis, in which infected animals show signs of encephalitis 6 days after infection, and approximately 50–70% of the animals succumb to infection. This animal model is an inexpensive, readily available means to study pathogenesis and test diagnostics, vaccines, and therapeutics.

A team of NIAID intramural researchers and collaborators from the Walter Reed Army Institute of Research has developed a hybrid vaccine that protects mice from WNV infection. The vaccine consists of a dengue virus backbone (proved to be safe in people) and select WNV genes. The hybrid vaccine stimulated strong immune responses against WNV after a single dose and protected all immunized mice from subsequent exposure to the New York WNV strain. The researchers are testing the vaccine in monkeys and hope to begin human trials in late 2002.

Primary Immunodeficiency Diseases

Chronic Granulomatous Disease (CGD)

CGD is an inherited genetic disorder characterized by failure of white blood cells (neutrophils) to produce the oxygenated compounds needed to kill microorganisms. NIAID intramural investigators completed a clinical trial in patients with CGD and demonstrated that itraconazole prophylaxis prevents fungal infections in these patients without significant toxicity.

X-Linked Agammaglobulinemia (XLA)

XLA, an inherited immunodeficiency disease, is characterized by reduced numbers of mature B cells, resulting in very low levels of antibodies. Many patients with XLA have mutations in the enzyme Bruton's tyrosine kinase (BTK). Using a mouse model, NIAID-supported investigators recently discovered a mechanism by which BTK mutations may lead to defects in antibody production. Normally, when the protein BRIGHT (B-cell regulator of immunoglobulin heavy-chain transcription) binds to DNA, antibodies are produced. When the *BTK* gene is abnormal, BRIGHT does not bind to DNA. The investigators showed that BTK associates with BRIGHT to form the DNA-binding complex that regulates antibody synthesis and that the physical association of these two proteins is essential for binding to DNA. When *BTK* is mutated, these complexes fail to form. This research defines a novel pathway that controls antibody synthesis.

X-Linked Lymphoproliferative Disease (XLP)

Patients with XLP, a primary immunodeficiency disorder, most often die from liver and bone marrow failure caused by inappropriate T-cell responses directed against the patient's own tissue. NIAID-supported investigators have discovered that the protein known as SAP regulates the differentiation of T cells into two distinct subsets, Th1 and Th2, which regulate each other and create a balance that is critical for normal immune responses. *SAP* gene mutation disrupts this balance, resulting in unregulated growth of Th1 cells and the tissue damage characteristic of XLP. This pathway is a potential target for novel therapeutic approaches for autoimmune and inflammatory diseases.

Other Immune System-Mediated Conditions

Autoimmune Lymphoproliferative Syndrome (ALPS)

ALPS is associated with inherited defect in programmed cell death of lymphocytes and manifested by chronic nonmalignant adenopathy and splenomegaly, as well as autoimmune conditions. ALPS patients have various immunological abnormalities, including circulation of multiple antibodies against the self and alterations in lymphocyte. NIAID researchers recently found that increases in circulating and lymphoid tissue interleukin-10 (cytokine) levels are associated with disease expression.

Cystic Fibrosis (CF)

CF is a genetically determined disease resulting from several variants in the CF transmembrane regulator *(CFTR)* gene. This gene is nearly always associated with chronic sinusitis and frequently occurs in asthma patients. CF occurs only when individuals inherit two mutant copies of the *CFTR* gene, one from each parent. NIAID-supported investigators discovered that individuals who inherit only a single copy of the mutant *CFTR* gene are highly predisposed to developing chronic sinusitis, suggesting that specific gene abnormalities are responsible for the development of chronic sinusitis in at least a subset of patients.

Graft Versus Host Disease (GVHD)

A complication of bone marrow transplantation or transplantation of hematopoietic stem cells (HSCs) derived from bone marrow is GVHD, in which T cells in transplanted HSC or bone marrow (the graft) attack the tissues of the transplant recipient (the host). NIAID investigators demonstrated in a mouse model that treatment with shortened or truncated antibodies against CD3 can prevent GVHD by triggering the death of donor T cells that can attack the host. These findings suggest that selective depletion of foreign T cells with the truncated anti-CD3 anti

bodies could be a potential strategy to prevent graft rejection.

An NIAID-supported investigator evaluated the efficacy of cord blood transplantation versus bone marrow transplantation in patients with acute leukemia, lymphoma, and aplastic anemia and demonstrated that recipients of cord blood HSC have a significantly lower risk of both acute and chronic GVHD than recipients of bone marrow HSC from identical siblings. These results suggest that cord blood transplantation is a safer and less expensive alternative to bone marrow transplantation for certain forms of leukemia and lymphoma.

Wegener's Granulomatosis (WG)

The underlying condition for WG, which is characterized by necrotizing granulomas and ulceration of the upper respiratory tract, is vasculitis of small vessels. NIAID investigators demonstrated that low-dose cyclophosphamide is a highly effective therapy for WG, and treatment regimens developed in this project are being used worldwide. However, long-term toxicities from this therapy have been observed in patients who have survived for many years after diagnosis. NIAID investigators recently demonstrated that methotrexate is an effective alternative to cyclophosphamide for certain patients with WG, and they are studying the long-term efficacy and toxicity of this drug.

Research Initiatives

Activities in Infectious Diseases

- The Bacteriology and Mycology Study Group (BAMSG) contract was awarded to the University of Alabama. This contract supports clinical evaluation of interventions for serious fungal and antibiotic-resistant bacterial infections. In addition, the Bacteriology and Mycology Biostatistical and Operations Unit contract was awarded to Rho Federal Systems Division, Inc. This contract provides data management and analysis, site monitoring, and operations support for clinical trials conducted by BAMSG, as well as studies conducted through the Clinical Studies of Chronic Lyme Disease contract.
- The Mycoses Study Group contract was supplemented and extended. A phase I study evaluating the anti-cryptococcal murine monoclonal antibody in patients who have recovered from AIDS-associated cryptococcal disease is open to enrollment and will be completed under this contract.
- NIAID awarded five new contracts under the RFP Animal Models of Human Viral Infections for Experimental Therapies. These new contracts will provide small animal models for both NIAID and the antiviral research community to be used to develop and evaluate new therapeutics for emerging and rare viral diseases. These small animal models mimic human diseases caused by herpes, orthopox, influenza, respiratory syncytial, and measles viruses.
- Through contracts with St. Louis University, Utah State University, and University of Alabama, NIAID provided funds for in vitro and in vivo screening of known antiviral compounds for activity against several RNA viruses of bioterrorism concern. To date, 350 compounds have been screened, and several compounds with activity against orthopoxviruses have been identified.
- A contract was awarded to The Institute for Genomic Research to support the Pathogen Functional Genomics Resource Center, which will offer the research community a wide range of genomic resources and technologies (e.g., relational databases and computational tools, microarrays, proteomics) for functional analysis of microbial pathogens and invertebrate vectors of infectious diseases.
- NIAID supports a pneumococcal reference and resource laboratory through a contract awarded to the University of Rochester. The laboratory develops and standardizes pneumococcal assays and reference reagents, measures and quantitates antipneumococcal antibody responses, develops new pneumococcal functional antibody assays, and disseminates antigens and reagents.

- Through the program announcement (PA) Therapeutics Research on AIDS-Associated Opportunistic Infections, NIAID awarded a grant for the study of antiviral drug resistance in human cytomegalovirus.
- Through an interagency agreement with the Office of Naval Research, NIAID funded work on the functional genomics and proteomics of *Bacillus anthracis* (etiologic agent of anthrax).
- NIAID continues to fund grants awarded in 2000 in response to the request for applications (RFA) Preparedness Against Illegitimate Use of Bacterial Pathogens. The projects address research in early events in pneumonic plague, host responses to tularemia, vaccine against Q fever, vaccine against *Brucella* (etiologic agent of brucellosis) and studies of its gene expression and virulence, and strategies for inactivating *Bacillus anthracis* spores and inhibiting cellular uptake of anthrax toxin.
- The RFA Preparedness Against Illegitimate Use of Bacterial Pathogens was reissued in 2001. Ten new grants were awarded for the following projects: improved vaccines against *Brucella* and the characterization of the *Brucella abortus* genetic element known as *virB* locus; vaccine against *Rickettsia* and sequencing of the *Rickettsia rickettsii* genome (etiologic agent of Rocky Mountain spotted fever); intracellular survival determinants of *Yersinia pestis* (etiologic agent of plague) and the structure of its type III export complex; analysis of *Burkholderia mallei* gene expression using the microarray technology; characterization of exosporium proteins and the role of hemolysins in the escape of *Bacillus anthracis* from immune cells (macrophages); and oral vaccine against multiple biowarfare agents.
- NIAID funded extramural projects on the hepatitis A, hepatitis D, and hepatitis E viruses. In addition, NIAID intramural investigators are characterizing the hepatitis A virus to engineer an attenuated virus vaccine that can be administered orally. They are manipulating the genome of the hepatitis A virus to increase the level of viral replication in cell culture, which will enhance vaccine production. They are also conducting primate studies to develop an appropriately attenuated hepatitis A virus strain. Research is ongoing to develop recombinant antibodies as an alternative to γ-globulin preparations currently used for immunoprophylaxis.
- NIAID provided supplemental funding for research on bacterial pathogens that could potentially be used as bioterrorism agents. Some of the projects that received supplemental funding are transport of *S*-adenosylmethionine in *Rickettsia*, actin-based motility of *R. rickettsii*, permeability of *R. prowazekii* (etiologic agent of epidemic typhus), vector biology and transmission of *R. typhi*, and iron transport and regulation in *Y. pestis*.
- Supplemental funds were provided for a research project on the use of the WNV hamster model to evaluate vaccines and antiviral agents.
- The Collaborative Antiviral Testing Group (CATG) is conducting in vitro screening of antiviral compounds with activity against viruses of potential bioterrorist threat, including the vaccinia, cowpox, yellow fever, Pichinde, Punta Toro, and Venezuelan equine encephalitis viruses. CATG is also evaluating compounds for in vitro activity against herpes, respiratory, hepatitis B, and West Nile viruses.
- CASG, which supports clinical trials of therapies for viral infections other than HIV, is evaluating the following pediatric protocols: the use of oral acyclovir following standard-of-care treatment with acyclovir to limit the recurrence of neonatal HSV infections limited to the skin, eyes, and mouth or to the central nervous system; comparison of oral valganciclovir to intravenous ganciclovir to treat symptomatic congenital CMV infections; and to treat neonatal enteroviral sepsis with pleconaril. In addition, CASG is evaluating four adult protocols, including intravenous ribavirin treatment of hantavirus pulmonary syndrome and valacyclovir for long-term therapy of herpes simplex encephalitis.

- NIAID provided supplemental funding from the NIH Director's Discretionary Fund for the following activities addressing transmissible spongiform encephalopathy (TSE): expansion of an ongoing contract with Utah State University to evaluate antiprion compounds in transgenic mouse models of human prion diseases, expansion of capabilities at the Centers for Disease Control and Prevention (CDC) for collection of potential TSE-infected materials, research on the mechanisms of transmission in elk with chronic wasting disease, and research to establish containment capabilities for handling TSE materials.
- NIAID continues to support grants awarded in response to a joint RFA with NIGMS—Evolution of Infectious Diseases. The projects focus on rabies, dengue, and enteric infections.
- NIAID supports several international programs to promote scientific cooperation on important infectious diseases and pathogens. The International Collaboration in Infectious Disease Research (ICIDR) program is designed to promote collaborative research between U.S. investigators and scientists in 15 countries where tropical infections are endemic. The ICIDR Opportunity Pool program supports pursuit of emerging research opportunities resulting from unexpected disease outbreaks or scientific advances. The Tropical Disease Research Units program is a domestic grants program for multiproject, interdisciplinary studies that seek to develop new strategies to control diseases caused by protozoa and helminths. The International Centers for Tropical Disease Research (ICTDR) program incorporates NIAID-supported tropical disease research centers into an interactive network focused on tropical infectious diseases.
- NIAID continues to collaborate with the Fogarty International Center to support the International Training and Research in Emerging Infectious Diseases program, which addresses the training needs for emerging and reemerging infectious diseases in developing countries.
- NIAID supported large-scale genome sequencing projects on microbial pathogens and invertebrate vectors of infectious diseases. Specifically, rare disease-related organisms included *C. immitis* (etiologic agent of coccidioidomycosis), group B streptococcus, *H. capsulatum* (etiologic agent of histoplasmosis), *R. rickettsii* (etiologic agent of Rocky Mountain spotted fever), *T. gondii* (etiologic agent of toxoplasmosis), *C. neoformans* (etiologic agent of cryptococcosis), *E. coli* O157:H7, *S. pneumoniae*, and *S. pyogenes*.
- With funds from the Defense Advanced Research Projects Agency, NIAID is sequencing the genome of several bacterial pathogens that have potential for use as bioterrorism agents. These *include R. typhi* (etiologic agent of endemic typhus), *Brucella suis* (etiologic agent of brucellosis), and *Burkholderia mallei* (etiologic agent of glanders).
- NIAID is supporting phase I and II trials for two candidate vaccines for human CMV: a glycoprotein subunit of CMV and engineered live recombinant viruses.
- NIAID supports a phase III efficacy trial of a 9-valent pneumococcal conjugate vaccine in West Africa. The trial is cosponsored by the United States Agency for International Development, The Gates Foundation, and the Medical Research Council in London. The trial is designed to determine the effect of coadministration of pneumococcal conjugate vaccine with DPT-Hib vaccine (Tetramune) on childhood mortality caused by invasive pneumococcal disease. Approximately 45,000 children will be recruited into the trial over 3.5 years.
- NIAID supports the Vaccine and Treatment Evaluation Units (VTEUs), which focus on phase I and phase II clinical trials of candidate vaccines. NIAID is sponsoring phase I safety trials of the following group A streptococcal vaccines: one consisting of live oral commensal bacterium; *S. gordonii*, serving as a vector for a conserved region of the M6 protein of *S. pyogenes*; and hexavalent vaccine consisting of a fusion protein contain

ing the M protein fragments from six serotypes. Also, awards were made for expanded phase II and IV vaccine trials in humans. The clinical trials implemented under this contract are phase II and IV vaccine immunogenicity and safety studies of investigational and licensed vaccines.

- NIAID supports The Streptococcal Initiative, which includes sponsoring a phase I safety trial of a group B streptococcal type V polysaccharide-tetanus toxoid conjugate vaccine and a phase II and III clinical trial of group B streptococcal type III polysaccharide-tetanus toxoid conjugate vaccine.
- NIAID intramural scientists are conducting a clinical protocol to evaluate, treat, and study patients with amebiasis and diseases caused by a variety of other gastrointestinal parasites. Participants are given the standard medical care and evaluated for immune response to infection and correlation between parasite burden and disease. Isolates from patients who do not respond to standard medications and dosing are studied in vitro to determine effective therapies.
- NIAID intramural investigators recently initiated a clinical study to identify genetic mutations responsible for chronic Epstein-Barr virus (EBV) infection and to learn more about its natural history. Knowledge gained from this study has the potential to provide insight into the immunological control of EBV infections.
- NIAID intramural scientists are conducting a cross-sectional study of patients with presumed chronic Lyme disease; those with chronic Lyme disease will be followed prospectively. The results will yield a prospective database from which diagnostic criteria can be established and future therapeutic trials can be designed. Other current intramural projects involve a new diagnostic procedure for Lyme disease based on the C6 peptide of the VlsE protein,; development of a new PCR assay for *Borrelia*, host immune responses and studies of magnetic resonance imaging patterns in Lyme disease, molecular mechanisms of adaptation and variation in *B. burgdorferi* and their roles in the infectious cycle, and diagnosis of Lyme disease by using recombinant DNA technology to clone *Borrelia* genes.
- NIAID has a large intramural program dedicated to understanding transmissible spongiform encephalopathy (TSE). Work includes studies of the mechanisms of TSE transmission and disease progression and the nature of the TSE infectious agent (prion), search for potential therapeutic compounds for TSE studies of the potential use of antibody-based and vaccine-based therapies for TSEs, and development of diagnostic tests for TSE.
- NIAID intramural researchers are analyzing the immune response to *Toxoplasma gondii* to define the pathways of host resistance. Goals are the design of strategies to protect humans against infection and elimination of the organism in immunocompromised individuals.
- NIAID intramural investigators and their international collaborators are conducting a clinical study of Peruvian patients with inactive cysticercosis (disease caused by encystment of tapeworm larvae). The study will determine the frequency and history of edema formation around calcified cysts, the frequency of associated symptoms, and the types of lesions and patients showing a propensity to develop edema around the lesion. Initial studies showed that approximately 35% of the patients had such edema. Investigators are instituting a therapeutic trial of a 14-day course of corticosteroids to see whether this treatment can decrease the duration of the edema and associated symptoms.

Activities in Primary Immunodeficiency Diseases

- Through a contract with the Immune Deficiency Foundation (IDF), NIAID maintains a registry of clinical information on U.S. residents affected by primary immunodeficiency diseases. For each disease, the registry collects information on clinical pheno

types and phenotype-genotype correlations, natural course of the disease including complications, effects of therapy, and causes of death. The diseases in the registry include CGD, X-linked hyper-IgM syndrome (XHIM), severe combined immunodeficiency disease (SCID), XLA, Wiskott-Aldrich syndrome, common variable immunodeficiency, leukocyte adhesion deficiency type I (LAD I), and DiGeorge syndrome.

- Patients with interferon (IFN)-γ receptor (IGR) deficiency have a defect in the genes for IFN-γ receptors found on certain immune cells. NIAID researchers are studying patients with IGR deficiency to develop gene transfer methods for treatment of the disease. Investigators are also working to improve the multidrug therapy that is currently the mainstay of treatment for patients with IGR deficiency.
- Children with XHIM lack or have only trace amounts of several classes of antibodies (e.g., IgG, IgA, IgE) and are plagued from infancy with severe, recurrent abscesses of the skin and lungs. This disease is caused by a defect in the T-cell surface molecule CD40L. NIAID researchers have initiated a study to treat XHIM patients with CD40L obtained from the Immunex Corporation. Patients will be given increasing doses of CD40L subcutaneously and then studied to determine whether their antibody levels and immune functions improve.
- LAD I is caused by the absence or diminished numbers of CD18 molecules required for the movement of certain white blood cells to inflamed areas in blood vessel walls where they promote healing. NIAID is conducting a clinical trial in which LAD I patients are given IFN-γ, which increases CD18 and cell migration to injured areas. This work will determine whether modest increases in CD18 or white blood cell movement could result in clinical improvement of LAD I patients.
- NIAID researchers are conducting a clinical study of hyperimmunoglobulin E syndrome patients and their relatives to better understand the medical problems associated with this disease, identify and treat complications, and identify responsible genes. They are looking for responsible genes by disrupting candidate genes in mice and to see whether the syndrome can be reproduced in this animal model.
- NIAID investigators are studying the cellular defects that cause CGD and are conducting two clinical trials with CGD patients. One trial involves using gene therapy to correct the cellular defects.
- SCID is a rare congenital syndrome characterized by little, if any, immune response. NIAID researchers developed retroviral-common γ-chain vectors and are collaborating with a group from the University of Pennsylvania to test the ability of these vectors to cure SCID in a dog model.

Activities in Other Immune System-Mediated Conditions

- NIAID supports 13 Asthma and Allergic Diseases Research Centers, which are the cornerstone of the pathobiology component of NIAID's asthma and allergy research program and provide support for basic and clinical research on the mechanism, diagnosis, treatment, and prevention of these diseases. Two of the centers are cofunded by NIEHS.
- NIAID supports the Immune Tolerance Network (ITN), an international consortium of more than 70 basic and clinical investigators, which conducts clinical trials to evaluate the safety and efficacy of tolerance induction strategies in clinical areas that include autoimmune and allergic diseases. ITN is cosponsored by NIDDK and the Juvenile Diabetes Research Foundation International (JDRF).
- NIAID supports four Autoimmunity Centers of Excellence (ACEs), which conduct collaborative basic and clinical research on autoimmune diseases, including single-site and multisite pilot clinical trials of immunomodulatory therapies. ACEs are currently enrolling participants in the trial of anti-CD20 therapy for systemic lupus erythema

tosus. ACEs are cosponsored by NIDDK, NIAMS, and ORWH.

- Through the Clinical Trials Network for Stem Cell Transplantation for Autoimmune Diseases, NIAID is developing clinical trials to assess the efficacy of hematopoietic stem cell transplantation in the treatment of several autoimmune diseases, including systemic lupus erythematosus and scleroderma.
- Through the RFA Hyperaccelerated Award/ Mechanisms in Immune Disease Trials, NIAID awarded four grants for mechanistic studies associated with clinical trials of immunotherapies for immune-mediated diseases, including autoimmune diseases.
- NIAID established the Autoimmune Diseases Prevention Centers to conduct basic research on new targets and approaches to prevent autoimmune disease and to evaluate novel approaches in pilot and clinical studies. These centers are cosponsored by NIDDK, NICHD, ORWH, and JDRF.
- Through the RFA Innovative Grants on Immune Tolerance, NIAID awarded 31 new grants to support research projects on the molecular mechanisms and applications of antigen specific immune tolerance. This initiative is cosponsored by NIDDK and NHLBI.
- The International Histocompatibility Working Group (IHWG) is a network of more than 200 laboratories in more than 70 countries that collect and share data on genes of the human leukocyte antigen complex. NIAID supports a project within IHWG to identify single nucleotide polymorphisms in immune response genes. These variations may account for the increased susceptibility of certain individuals or groups to immune-mediated diseases.
- NIAID is supporting the Multiple Autoimmune Disease Genetics Consortium, a repository of genetic and clinical data and materials from families in which two or more individuals are affected by two or more distinct autoimmune diseases. This resource provides materials to promote research aimed at discovering human immune response genes involved in autoimmunity. To date, 121 families have been enrolled. More information can be found at www.madgc.org/.
- Mastocytosis is a disease caused by abnormal proliferation of mast cells in various tissues. NIAID intramural scientists are trying to identify mutations in genes regulating mast cell growth and differentiation. They are also seeking novel markers of disease activity. The findings will be correlated with clinical patterns of disease and prognosis.

Newly Issued Initiatives

- Drug Development for Opportunistic Infections requests for proposals (RFPs): These contract resources will allow NIAID to support investigator-initiated drug discovery, stimulate private-sector sponsorship of new drugs, and provide information for selection of antiopportunistic infection drug candidates for clinical studies. The organisms of focus include *Mycobacterium avium* complex, *Pneumocystis carinii*, *Cryptosporidium parvum*, and *Cryptococcus neoformans*.
- Preparedness Against Illegitimate Use of Bacterial Pathogens: This initiative was first issued in 2000 and reissued in 2001. The goal is to support research on molecular and genetic aspects of virulence and pathogenesis and on vaccine strategies against bacterial pathogens that may be used in biological warfare. Many of these pathogens cause rare diseases.
- Infectious Etiology of Chronic Diseases: Novel Approaches to Pathogen Detection: The goal of this initiative is to encourage research on technologies to identify and validate the role of microbial pathogens in chronic diseases and cancer for which an infectious etiology is suspected. This RFA is cosponsored by NCI, NIDDK, and ORWH.
- Therapeutics Research on AIDS-Associated Opportunistic Infections and Malignancies: The goal is to stimulate preclinical research for novel therapeutic strategies against opportunistic infections and malignancies in people with HIV/AIDS. The AIDS-associated

opportunistic organisms emphasized in this PA include *Pneumocystis carinii*, *Cryptosporidium parvum*, and microsporidia. This PA is jointly sponsored by NIAID, NCI, and NIDCR.

- Prevention of Group B Streptococcal Disease: This RFA is to recompete the 5-year collaborative multidisciplinary research contract awarded in 1997 to the Channing Laboratory, Brigham and Women's Hospital. Its major focus is clinical studies in select populations to further understand GBS infection and studies of the host immune response.

Cooperative Research and Development Agreements (CRADAs)

- NIAID funded three new CRADA projects related to rare diseases: analysis of gene expression in T cells as it relates to immune regulation in organ-specific autoimmune diseases, development and animal testing of rabies vaccines, and the study of allogeneic stem cell transplantation for CGD.
- NIAID continued to support CRADAs from previous years. The projects address ex vivo stem cell therapy for CGD, hepatitis E vaccine, production and clinical evaluation of the anti-HSV antibody for treatment of neonatal HSV infections, adult pertussis vaccine, identification of varicella-zoster gene targets, and an efficacy trial of a pneumococcal conjugate vaccine.

Workshops, Symposia, and Meetings

- NIAID held the workshop The Role of Innate Immunity in the Etiopathology of Autoimmune Disease. It was attended by physicians and scientists, and cofunded by ORD, ORWH, and the American Autoimmune Related Diseases Association (AARDA).
- NIAID held the meeting titled Class II MHC Gene Control and Disease Relevance, which brought together basic and clinical researchers who had complementary interests in MHC (major histocompatibility complex) class II gene expression and the disease manifestations of aberrant or impaired expression (e.g., bare lymphocyte syndrome). Bare lymphocyte syndrome is a rare, congenital disorder with clinical manifestations similar to SCID. This meeting reviewed and updated the state of science and examined opportunities for collaboration between basic and clinical investigators.
- NIAID hosted the Imaging Technology and Study of Immune Function Workshop for NIAID-supported investigators funded under the research initiative New Imaging Technologies for Autoimmune Diseases. These investigators will identify emerging areas of opportunity for imaging technologies in the study of the immune system, particularly in regard to autoimmunity and transplantation.
- NIAID sponsored the 10-year anniversary meeting of the ICTDR network, which consists of NIAID-supported institutions and centers focused on research in tropical diseases and includes approximately 20 international research sites in approximately 15 countries. The workshop included sessions on diagnostics, pathogenesis, vector research, drug targets and treatments, and vaccines.
- NIAID held the 36th Joint Conference on Parasitic Diseases (U.S.–Japan Cooperative Medical Science Program). This program focused on vector biology, helminth diseases, and *Entamoeba histolytica.*
- NIAID hosted the international workshop Intranasal Administration of Toxin Adjuvants. Preclinical and clinical data on the intranasal administration of cholera toxin, *E. coli* heat-labile toxin, and mutants derived from them were reviewed. These molecules are excellent adjuvants that induce strong mucosal immune responses when administered orally or intranasally.
- NIAID held the workshop Viral Mechanisms of Immune Evasion to define research opportunities and gaps and to formulate a research agenda in this research area.
- NIAID collaborated with NINDS and ORD to hold a conference on neuroborreliosis.

The primary goal of the conference was to encourage productive interactions and facilitate the exchange of new information among investigators conducting controlled clinical studies on chronic Lyme disease.

- With funding from ORD, NIAID held five meetings that focused on the development of *Flavivirus* vaccines, animal models of autoimmune disease, gene therapy for primary immunodeficiency diseases, bare lymphocyte syndrome, and the role of innate immunity in autoimmune diseases.

National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)

Overview

The mission of NIAMS is to support basic and clinical research into the causes, treatment, and prevention of arthritis and musculoskeletal and skin diseases; train scientists to carry out this research; and disseminate information on research progress in these diseases. Basic research involves various scientific disciplines, including immunology, genetics, molecular biology, biochemistry, physiology, virology, and pharmacology. Clinical research comprises rheumatology, orthopedics, bone endocrinology, sports medicine, dermatology, and aspects of pediatrics and gerontology related to arthritis and musculoskeletal and skin diseases.

Recent Scientific Advances

Lupus

Lupus is a serious and potentially fatal autoimmune disease that often occurs in women of child-bearing age—nine times more women than men have lupus. Lupus occurs in all races; however, African American women have incidence and mortality rates three times higher than white women and tend to develop the disease at a younger age with more serious complications. Lupus is also more common in women of Hispanic, Asian, and Native American descent than in white women. The manifestations of lupus are diverse, affecting many parts of the body including joints, skin, kidneys, heart, lungs, blood vessels, and brain. NIAMS-supported researchers have focused on involvement of the nervous system in some people with lupus and have reported significant advances in understanding of the molecular mechanisms involved in changes that can occur in the brain of people with lupus. These researchers reported that the DNA-attacking antibodies in people with lupus may also attack molecules that bind a particular neurotransmitter (glutamate) involved in nerve cell activity and can cause death of the nerve cells. These antibodies are present in cerebrospinal fluid, thereby possibly affecting brain function. Although researchers had previously documented cognitive dysfunction in some patients with lupus, the mechanism involved was unclear. This new research finding not only clarifies the nervous system complications associated with lupus, it also presents new therapeutic possibilities for these aspects of lupus that can be challenging for patients, their families, and their health-care providers.

Marfan Syndrome

Marfan syndrome is a heritable condition that affects connective tissue. Individuals with Marfan syndrome tend to be thin and have excessively long bones. Other manifestations include skeletal malformations, abnormal position of the lens of the eye, and enlargement of the beginning of the aorta. If left untreated, an enlarged aorta can lead to hemorrhage and even death. This disorder results from mutations in the gene that makes fibrillin-1 (FBN1), a major component of the elastic fibers surrounding blood vessels. NIAMS-supported studies on tissue analysis of mouse models recently refined understanding of what leads to vascular disease associated with Marfan syndrome. The results indicate that, contrary to previous belief, the progression of Marfan syndrome is not caused by failure of formation of an elastic fiber network but rather because the network that forms is defective. These findings have implications for the possible future design of gene therapy for Marfan syndrome. Expanded studies to genetically engineer mice with increased longevity would allow study of the development and progress of aortic aneurysms in general and those associated with Marfan syndrome in particular and could lead to the development of new therapies that would enable their prevention.

Osteoarthritis

Osteoarthritis is the most common joint disease. Also known as degenerative joint disease, osteoarthritis occurs when cartilage begins to fray,

wear, and decay, causing joint paint, reduced joint motion, and loss of joint function and disability. As the number of older people in the population increases, osteoarthritis will affect a greater proportion of the American public. NIAMS partnered with NIA to establish a public-private partnership that will develop clinical research resources to support discovery and evaluation of biomarkers and surrogate endpoints for osteoarthritis clinical trials. After several years of effort, NIAMS and NIA recently joined with other NIH components, including NIDCR, NCCAM, ORWH, NCMHD, other Federal agencies, and four pharmaceutical companies in funding the newly launched Osteoarthritis Initiative. For the first time, a public-private partnership will bring together new resources and commitment to help find biological markers for the progression of osteoarthritis. The Osteoarthritis Initiative will fund four to six clinical research centers to establish and maintain a natural history database for osteoarthritis (including clinical evaluation data and radiological images) and a biospecimen repository. All data and images collected will be available to researchers worldwide to quicken the pace of scientific studies and biomarker identification.

Osteoporosis

Osteoporosis remains a significant public health challenge, particularly among the elderly. Reports from the Framingham osteoporosis study recently provided new information on the effect of dietary protein on bone loss in elderly men and women. This is an important study regarding the role of diet (particularly protein nutrition) on bone health maintenance in the aging population. Results from this study should encourage adequate consumption of dietary protein in the elderly population and demonstrate the potential benefits of this dietary change on skeletal health.

In addition, researchers have reported new insights into the complex effects of estrogen on bone. Bone breakdown, or resorption, is a normal part of bone remodeling, during which old or damaged bone is replaced with new bone. Two recent reports from NIAMS-supported researchers have provided important clues to the complex relationship between estrogen and bone and have demonstrated that much is still to be learned about the action of estrogen and the function of estrogen receptors. In the most surprising development, investigators have extended earlier work showing that estrogen decreases rates of controlled cell death (apoptosis) among bone-forming cells (osteoblasts), thus increasing bone formation and preventing net bone loss. Now they find that either estrogen or androgen can have this antiapoptotic effect and that it can be mediated by either estrogen receptors or androgen receptors, regardless of which sex hormone is present. The effects of sex hormones on bone seem to reflect a previously unrecognized function of the estrogen and androgen receptors that is distinct from their familiar action on reproductive tissues. In a second study, investigators showed that T cells can contribute to the bone loss that occurs when estrogen levels are low through the stimulation of osteoclasts that resorb bone, thereby enhancing the susceptibility to fractures.

Osteogenesis Imperfecta

Genetic diseases are caused by mutated genes that either produce abnormal proteins or fail to produce any protein at all. Osteogenesis imperfecta, one of the most common genetic bone diseases, is caused by mutations in the gene for type I collagen. NIAMS-supported investigators have recently reported progress in both the controlled introduction of genes into bone cells and the design of agents for inactivating disease-causing mutant genes.

Pseudoxanthoma Elasticum (PXE)

PXE is a systemic inherited disorder that involves the elastic tissue in the skin, eyes, and cardiovascular system. It can result in severe and even fatal health problems or may be much milder and clinically difficult to identify unless suspected and pursued vigorously. A consortium of investigators localized the gene underlying PXE a little over a year ago. This gene encodes for a protein that underlies multiple drug resistance in microorganisms but appears to transport materials through the membrane of human cells. In a recent study, affected individuals from four families were investigated to determine the spe

cific genetic defects underlying the disease in their particular family. The four families were from different ethnic backgrounds, yet the specific defect in all four families was identical. This common defect appears to be a genetic hot spot and suggests that the same mechanism of mutation leads to this uniformity of mutation in different families. Recognizing that this is a metabolic disease offers hope for the development of treatment based on metabolic modifications, which could potentially include, for example, diet manipulation or drug therapy. Isolating the gene and cataloging the gene defects underlying the disease, particularly in families in which one member has been identified, will enable early identification of affected individuals so that treatment can be instituted before signs or symptoms develop and, hopefully, early enough to prevent these symptoms from developing.

Psoriasis

Psoriasis is a common and chronic skin disease characterized by scaling and inflammation. It affects millions of Americans of all ages. NIAMS-supported scientists recently evaluated the efficacy of a new topical vitamin D derivative and found a significant decrease in psoriasis severity. New treatments for psoriasis, including cyclosporin A and other therapies, modulate immune system function. Progress is also being made in identifying genes linked to psoriasis. Scientists have determined that, at least in some forms of hereditary psoriasis, a linked gene is located on chromosome 17q. Once the specific genes for psoriasis have been isolated and their products determined, greater insight into the disease process will be available, opening new avenues for intervention and improving treatment for psoriasis.

Activities and Initiatives

Duchenne Muscular Dystrophy (DMD)

DMD is a genetic muscle-wasting disease caused by mutations in the gene that codes for the protein dystrophin. As a result of the spring 2000 workshop on therapeutic approaches for DMD, NIAMS and the NINDS cosponsored a program announcement (PA) on therapeutic and pathogenic approaches for muscular dystrophies, including DMD. NIAMS recently made several new awards, including one for a project designed to identify the specific immune cells involved in DMD, which may point to potential immune-based therapies.

Based on the spring 2000 workshop in inflammatory myopathy, NIAMS, NINDS, and NIDCR are cosponsoring a PA on the pathogenesis and treatment of inflammatory muscle disease. Because of need, this initiative will encourage studies on inflammatory myopathies and the role of inflammation and inflammatory cells in muscular dystrophy.

Ehlers-Danlos Syndrome (EDS)

EDS is a set of conditions characterized by hyperextensibility of the skin, easy bruisability, increased joint mobility, and abnormal tissue fragility. NIAMS is committed to developing and disseminating science-based health information for patients and families affected by heritable disorders of connective tissue, including a new questions-and-answers fact sheet that includes information on EDS.

Facioscapulohumeral Muscular Dystrophy (FSHD)

FSHD is the third most common genetic disease of skeletal muscle. NIAMS and NINDS are implementing recommendations from the spring 2000 research planning conference on FSHD. Stemming from the conference was a request for applications (RFA) on exploratory research on FSHD. As a result, NIAMS and NINDS recently awarded six new grants to support both basic and clinical research studies on FSHD. In fall 2000, NIAMS and NINDS funded a national research registry for FSHD and myotonic dystrophy (DM). The long-term goal of the registry is to facilitate research in FSHD and DM by serving as a liaison between families affected by these diseases who are eager to participate in specific research projects and investigators interested in studying these disorders. Active recruitment of patients with FSHD and DM began in fall 2001.

Fibrous Dysplasia

Fibrous dysplasia of bone is a deforming and crippling skeletal disorder characterized by softening and bending of bone because of failure of bony tissue to calcify. Fibrous dysplasia is a feature of complex syndromes, such as McCune-Albright, although it occurs in clinically distinct contexts as well. Several components of NIH support research related to fibrous dysplasia. Recently, colleagues at NIDCR and NIDDK discovered that mutations in the protein Gs-α are involved in McCune-Albright syndrome, as well as other conditions. Basic biology researchers are beginning to study the function of Gs-α in the skeleton. and NIAMS currently supports two such projects. One is using genetically altered mice to explore the role of Gs-α in mediating signals that control cartilage and bone growth. The second is studying patients with progressive osseous heteroplasia (which includes a component of fibrous dysplasia) to test the hypothesis that the disease is caused by mutations in the gene for Gs-α. Results from these studies may lead to better therapies for patients affected by fibrous dysplasia.

Health Partnership Program

NIAMS has devised and implemented a unique strategy, the Health Partnership Program, to address health disparities. In the first phase of this program, NIAMS implemented a model community-based research program to study rheumatic diseases among the African American and Hispanic/Latino communities in metropolitan Washington, D.C.

In addition, through collaborations with community leaders, NIAMS created and opened the new NIAMS Community Health Center in July 2001 in a medically underserved area of Washington, D.C. This center gives researchers the opportunity to 1) increase understanding of health disparities in rheumatic diseases, 2) provide health care to the community, 3) increase minority participation in research studies, 4) increase the number of underrepresented biomedical researchers, and 5) train NIAMS rheumatology fellows to care for patients from minority communities. Patients are seen under the Natural History of Rheumatic Diseases protocol, which is conducted by investigators from the NIAMS intramural research program. Researchers expect to develop related health disparities studies based on outcomes of the Natural History protocol. In addition, health education is an important component of the program and includes developing brochures and fact sheets and conducting arthritis education seminars.

Juvenile Rheumatic Diseases

NIAMS is developing a national Pediatric Rheumatology Research Network to foster cooperation among researchers in the Institute's intramural program and extramural investigators committed to pursuing new studies in this field. The network will promote coordination of research efforts and resource sharing across institutions. NIAMS is also working to provide research training for pediatric rheumatologists to develop a cadre of individuals trained in research methods and clinical practice.

The Institute recently funded a new core center to strengthen understanding of the causes of and find novel approaches to treating pediatric rheumatic diseases. The center has five components: a repository to make tissues available to researchers, magnetic resonance imaging to monitor disease progression, identification of cells involved in rheumatic diseases, data processing and bioinformatics, and administrative support to coordinate project activities.

NIAMS is funding a research registry for juvenile rheumatoid arthritis, which serves as a national resource for scientists studying pediatric rheumatic diseases such as juvenile forms of arthritis. Finally, NIAMS is committed to disseminating science-based health information for patients and families affected by juvenile arthritis. To this end, the Institute has recently updated its questions-and-answers fact sheet on juvenile rheumatoid arthritis.

Lupus

NIAMS recently funded a new multidisciplinary clinical research center to study cardiovascular disease in rheumatic conditions such as lupus. Cardiovascular disease is a major complication for people with lupus, and African Americans in particular are at greater risk for both diseases, separately and as combined illnesses. Researchers will evaluate the effects of aggressive therapy in preventing cardiovascular complications in people with lupus in an effort to develop an effective disease management program. In addition, the Institute released an RFA on neuropsychiatric systemic lupus erythematosus to stimulate additional studies of the neurological and psychiatric syndromes, including cognitive, behavioral, affective, and motor manifestations, associated with this chronic disease.

The Institute's intramural research program has recently begun a study to increase understanding of the progression and natural history of rheumatic diseases such as lupus in minority communities. Intramural scientists are also examining the effects of Thunder God vine, a Chinese herb, on patients with autoimmune diseases such as lupus.

The Institute partnered with the National Medical Association to present a plenary discussion at the National Medical Association's 2001 Annual Convention and Scientific Assembly on the impact of lupus in the African American community. This session was designed to explore all aspects of lupus as it relates to bench-to-bedside research, including genetics, clinical aspects, pregnancy, outcomes of lupus, and lupus and cardiovascular disease.

To improve communication about lupus, NIAMS recently published *The Many Shades of Lupus: Information for Multicultural Communities*. This booklet, geared toward patients and their families, provides practical information about lupus signs and symptoms, disease management, and current research.

Marfan Syndrome

As a result of the Third Workshop on Heritable Disorders of Connective Tissue, held in fall 2000, NIAMS issued an RFA for basic and clinical research on heritable disorders of connective tissue. NIAMS is also committed to disseminating science-based health information for patients and families affected by connective tissue disorders. To this end, NIAMS has recently developed a questions-and-answers booklet on Marfan syndrome and a booklet on heritable disorders of connective tissue, which includes information on conditions such as Marfan syndrome.

Osteogenesis Imperfecta

NIAMS has undertaken several initiatives in osteogenesis imperfecta. In FY 2001, NIAMS issued an RFA targeting new research strategies. This solicitation built on recommendations made at the scientific meeting in September 1999, cosponsored by NIAMS, ORD, the Osteogenesis Imperfecta Foundation, and the Children's Brittle Bone Foundation to identify ways to expand the scope of osteogenesis imperfecta research. As a result of the request for applications, NIAMS funded five new grants to support research activities ranging from cutting-edge gene and cell therapies to testing drug treatments in animal models.

Paget Disease

Paget disease is a chronic disorder that typically results in enlarged and deformed bones. The excessive breakdown and formation of bone tissue that occurs in Paget disease can cause bones to weaken, resulting in bone pain, arthritis, deformities, and fractures. Current research is seeking to develop an animal model of Paget disease by introducing viruses or expressing viral genes in mice. Genetic research has linked Paget disease to chromosome 18q, and through grant awards from NIAMS, researchers are investigating the possibility of the involvement of multiple genes in the predisposition to the disease. Also, several researchers are investigating the occurrence of osteosarcoma in individuals with and without Paget disease to evaluate the presence of a genetic link. Osteosarcomas are believed to result from a series of genetic alterations that transform osteoblasts into a malignant state. Research addressing a genetic link between pagetic osteosarcoma and sporadic osteo

sarcoma will enhance future development of treatments for both diseases.

Scleroderma

Scleroderma is a group of diseases involving the abnormal growth of connective tissue, which supports the skin and internal organs. Scleroderma may affect only the skin, making it hard and tight, or it may also damage blood vessels and internal organs, such as the heart, lungs, and kidneys. Following up on a fall 2000 request for applications, NIAMS and ORWH recently awarded 10 grants that will explore the progression of scleroderma and new therapeutic approaches. The Institute is also supporting development of a national scleroderma family registry and DNA repository. The overall objective is to identify genes that influence susceptibility to scleroderma.

NIAMS currently supports two centers specializing in scleroderma research where scientists have found that fibroblasts may contribute to scleroderma and that both genetic and environmental factors appear to influence disease susceptibility and progression. One of these centers, recently funded through a special solicitation, is conducting research on biological processes that cause progression of scleroderma. Gaining better understanding of these biological processes could lead to new treatment approaches. In addition to funding a number of projects on autoimmunity and autoimmune diseases such as scleroderma, NIAMS has recently published a health booklet on scleroderma intended for, affected patients, their families, and the public.

Sjögren Syndrome

Sjögren syndrome is an autoimmune disease in which the immune system targets moisture producing glands and causes dryness in the mouth and eyes. The disease can affect other glands as well, such as those in the stomach, pancreas, and intestines, and can cause dryness in other places that need moisture, such as the nose, throat, airways, and skin. Patients with Sjögren syndrome often experience dry skin, rashes, and joint and muscle pain. NIAMS supports several research projects on Sjögren syndrome, including studies to better understand the molecular basis of immune system abnormalities in diseases such as Sjögren and investigations to identify genes that govern the propensity to develop autoimmunity. These efforts should lead to the development of more effective therapies.

The Institute is committed to developing and disseminating science-based health information on Sjögren syndrome and other autoimmune diseases. To this end, NIAMS recently developed fact sheets in Spanish on "El Síndrome de Sjögren" to complement the English version of this publication.

Trans-NIH Efforts in Musculoskeletal Medicine

NIAMS recognizes the opportunity to make a significant difference in the field of musculoskeletal medicine. In the spirit of focusing expertise in bone and cartilage biology, NIAMS joined with NICHD, NCCAM, NIMH, and NINDS in focusing resources in this area in trans-NIH efforts. This new effort will be located across from the NIH campus at the National Naval Medical Center in a renovated set of buildings encompassing both basic and clinical research space. This trans-NIH collaboration will build on existing strengths that are beginning to be coordinated, enhance research productivity through synergy of the programs, develop new programs, recruit new investigators, coordinate with existing and newly developed clinical programs, and make it possible to create a national resource in this critical and underserved area of research. Diseases to be addressed include osteoarthritis, osteoporosis, fibrous dysplasia, osteogenesis imperfecta, low back pain, temporomandibular joint disorder, and genetic diseases of cartilage and bone.

National Cancer Institute (NCI)

Overview

Cancer, in general, is not a rare disease; it is the second leading cause of death in the United States and accounts for one in every four deaths. In 2002, more than 550,000 Americans are expected to die of cancer—an average of more than 1,500 people per day. Despite reductions in age-adjusted rates of cancer death in recent years, the total number of recorded cancer deaths in the United States continues to increase, largely because of an aging and growing population.

Although cancer is not a rare disease, it is actually many distinct diseases, most of which are still considered rare. Only breast, prostate, lung, skin, and colon cancers can no longer be classified as rare because their prevalence exceeds the 200,000 cases per year maximum that defines rare diseases. Unfortunately, incidence and mortality rates have risen for many rare cancers, including cancers of the esophagus, liver, kidney, and brain, as well as melanoma, non-Hodgkin lymphomas, and multiple myeloma.

NCI's goal is to stimulate and support scientific discovery and its application to achieve a future in which all cancers are uncommon and easily treated. NCI works toward this goal in two main ways:

- It provides vision to the nation and leadership for NCI-funded researchers across the United States and around the world.
- It works to ensure that the results of research are used in public health programs and clinical practice to reduce the burden of cancer for all people.

Recent Advances

Cancer Biology and Etiology

Basic research studies that explore the mysteries of how cancer develops form the foundation of cancer research. Through these studies, scientists are identifying, at the molecular level, fundamental processes that underlie a cell's transformation from normal to malignant. Identifying the processes and pathways that lead to cancer provides attractive targets for new prevention and treatment approaches. Likewise, elucidation of the external and internal factors that cause or contribute to cancer opens avenues for developing behavioral interventions and drugs to prevent cancer.

Kaposi Sarcoma Herpesvirus (KSHV)

Kaposi sarcoma is an aggressive and disseminated cancer of the lungs, gastrointestinal tract, and lymph nodes that is frequently seen in patients with human immunodeficiency virus (HIV)/AIDS. KSHV is etiologically linked to all forms of Kaposi sarcoma and to rare primary effusion lymphomas in patients suffering from AIDS. In the early stages of any viral infection, there is a race between the infecting pathogen and the host's immunological defenses. NCI-supported scientists have found that the virus expresses two novel viral proteins early in KSHV infection that interfere with the immune system's ability to recognize and destroy the infecting KSHV virus, thus allowing KSHV to successfully evade the host's immunological defenses. KSHV evasion of the immune system is an important aspect of the virus's ability to infect susceptible hosts and establish a lifelong latent infection, leading to viral disease pathogenesis and oncogenic sequelae such as Kaposi sarcoma. Understanding the mechanisms of immune evasion may lead to prevention of KSHV infection and its neoplastic complications.

EWS/FLI Transcription Factor and *PDGF-C* Gene

Various human malignancies are associated with aberrant transcription factors—important components of the cell's machinery that regulate gene expression and protein synthesis. The childhood tumors Ewing sarcoma and peripheral primitive neuroectodermal tumor are characterized by a chromosomal translocation resulting in expression of the chimeric transcription factor EWS/FLI. The key to understanding the biology

of tumor-related transcription factors is to elucidate their gene targets. An NCI-supported scientist has used an innovative assay to identify a gene targeted by EWS/FLI—the recently identified platelet-derived growth factor C (*PDGF-C*). Expression of this growth factor in Ewing family tumor (EFT) cell lines and primary tumors depends on EWS/FLI activity. Moreover, dominant negative *PDGF-C* inhibits the growth of EFT cells. These results suggest that *PDGF-C* may be a significant mediator of oncogenesis in the sarcomas and that agents that block *PDGF*-receptor signaling may also inhibit *PDGF-C*-induced transformation.

Neuroblastoma and Activation of Apoptosis Pathway

Neuroblastoma is the most common extracranial solid tumor of childhood. Despite major advances in cancer chemotherapy and the use of bone marrow transplantation, the long-term survival rate for neuroblastoma patients with poor biological features and stage 4 (metastatic) disease remains less than 10%. The process of apoptosis in high-risk neuroblastoma is defective and may contribute to both the genesis and progression of this disease. An NCI-supported investigator has determined that antiapoptotic factors are highly expressed in neuroblastoma tumors, particularly those with other poor prognostic features such as amplification of the *MYCN* oncogene. This laboratory has also found that expression of antiapoptosis factors in neuroblastoma cell lines confers resistance to chemotherapy and that one such factor, Bcl-2, cooperates with *MYCN* to potentiate the malignant phenotype of neuroblastoma. More recently, this laboratory has studied the mechanism by which these neuroblastoma cells die in response to cytotoxic agents commonly used to treat neuroblastoma. These studies suggest that cisplatin and doxorubicin engage different cell death pathways to kill neuroblastoma cells and, specifically, that activation of the transcription factor NF-κB mediates doxorubicin-induced cell death. These studies could lead to additional therapeutic agents targeting NF-κB function in the diseased cells.

Birt Hogg Dube Syndrome (BHD)

Researchers have discovered that the risk of developing renal tumors and spontaneous pneumothorax was elevated in individuals with BHD compared with the risk in unaffected siblings. They have also determined the chromosomal location of the *BHD* gene and have described the spectrum of renal tumors associated with BHD.

Animal Model for Burkitt Lymphoma (BL)

Scientists have developed a mouse model for BL, a solid tumor disease of the B lymphocytes. The genetically engineered mouse is the first to develop lymphomas with striking similarities to human BL. The new mouse model may enhance understanding of the molecular and genetic components of this cancer and other lymphomas and permit testing of new treatments for BL.

Link Between DNA Repair and Cancer

NCI investigators are studying the role of DNA repair in rare human cancer-prone genetic diseases, primarily xeroderma pigmentosum (XP), Cockayne syndrome, and trichothiodystrophy. These researchers used XP cells to learn about the role of DNA repair in immunity and in the anticancer activity of ecteinascidin 743 isolated from sea squirt. They also found that a common variant of a DNA repair gene is associated with increased cancer risk. While characterizing clinical and laboratory abnormalities in XP, Cockayne syndrome, and Werner syndrome, the scientists also discovered new clinical disorders associated with these abnormalities.

Cellular Telephone Use and Brain Cancer

NCI scientists included cell phone use as part of a comprehensive study on the causes of brain tumors that began in 1994. The ongoing NCI adult brain tumor study involves about 800 adult brain tumor cases and 800 control subjects (people without brain tumors) from three medical institutions in Phoenix, Boston, and Pittsburgh. NCI researchers found that people who use cellular phones did not have a higher risk of brain tumors than nonusers. The researchers found no evidence that a person's risk of developing a brain tumor increased with increasing years of use or average minutes of use per day, nor did

brain tumors among cellular phone users tend to occur more often than expected on the side of the head on which the person reported using their phone. The NCI study began in 1994 and was completed in 1998, during a time when analog phones were primarily used. Digital phones, which operate at a higher frequency, are more commonly used today. However, no evidence at this time suggests that cancer risk would differ for the two types of phones.

Squamous Cell Esophageal Cancer Among U.S. Black Men

Data from a population-based case-control study were used to evaluate the relation between social class factors and squamous cell esophageal cancer and the extent to which alcohol, tobacco, diet, and low income contribute to the higher incidence among black than among white men in the United States. Patients, ages 30–79, with esophageal cancer histologically confirmed between 1986 and 1989 were selected from three U.S. geographic areas (Atlanta, Detroit, and New Jersey). The combination of all four major risk factors—low income, moderate/heavy alcohol intake, tobacco use, and infrequent consumption of raw fruits and vegetables—accounted for almost all of the squamous cell esophageal cancers in whites (98%) and blacks (99%) and for 99% of the excess incidence among black men. The study concluded that lifestyle modifications, especially a lowered intake of alcoholic beverages, would markedly decrease the incidence of squamous cell esophageal cancer in both racial groups and would narrow the racial disparity in risk. Further studies on the determinants of social class may help identify a new set of exposures for this tumor that are amenable to intervention.

Nutrient Intake and Esophageal and Gastric Cancers

In a population-based case-control study in Connecticut, New Jersey, and western Washington State, higher intake of nutrients found primarily in plant-based foods was associated with a reduced risk of adenocarcinomas of the esophagus and gastric cardia, whereas higher intake of nutrients found primarily in foods of animal origin was associated with an increased risk.

Diet/Nutrition and Multiple Myeloma Among Blacks and Whites

To explore whether dietary factors contribute to the risk of multiple myeloma and the twofold higher incidence among blacks compared with whites in the United States, data were analyzed from a population-based case-control study of multiple myeloma in three areas of the United States. Elevated risks in blacks were associated with obesity versus normal weight, whereas the frequency of obesity was greater for black than white control subjects. Reduced risks were related to frequent intake of cruciferous vegetables and fish in both races combined. Thus, the study concluded that the higher frequency of obesity among blacks may explain part of the higher incidence of multiple myeloma among blacks in the United States. In addition, the increasing prevalence of obesity in both blacks and whites may have contributed to the upward trend in incidence of multiple myeloma during recent decades.

Obesity, Hypertension, and Kidney Cancer in Men

In the United States, renal cell carcinoma is among the most rapidly increasing types of tumors in incidence, particularly among blacks. To clarify the association of obesity and hypertension with renal cell cancer, NCI scientists conducted a longitudinal study of Swedish construction workers and found that elevations in body mass index (BMI) and blood pressure independently increased the long-term risk of renal cell cancer. Reductions in BMI and blood pressure were found to lower the risk. These findings suggest that effective control of weight and hypertension may be useful in preventing this increasingly common cancer.

Pancreatic Cancer

A case-control study based on direct interviews was untertaken to estimate the effects of speculative risk factors, such as diet/nutrition and alcohol consumption, and those of established risk factors, such as cigarette smoking, diabetes mel

litus, and family history of pancreatic cancer, on pancreatic cancer risk. The investigation was population based in Atlanta, Detroit, and 10 New Jersey counties from August 1986 through April 1989. The study revealed a significant interaction between BMI and calorie intake that was consistent by both race and gender. Subjects with elevated BMI and calorie intake had increased risk, whereas those with elevated values for one of these factors but not the other experienced no increased risk. This finding suggests that energy balance may play a major role in pancreatic carcinogenesis. Diabetes mellitus was also a risk factor for pancreatic cancer and a possible complication of the tumor. The data were also consistent with a key role for hyperinsulinemia, particularly among nondiabetic individuals with elevated BMI. A threefold risk of pancreatic cancer was apparent among first-degree relatives of affected individuals. Increased risk was also associated with a family history of colon, endometrial, ovarian, and breast cancer, suggesting a possible link to hereditary nonpolyposis colon cancer. The findings supported a causal role for cigarette smoking; however, alcohol consumed at levels typical for the general U.S. population did not appear to be a risk factor, although heavy drinking may be related to risk, particularly in blacks.

Helicobacter Pylori and Pancreatic Cancer

The association of *Heliocobacter pylori* carriage and exocrine pancreatic cancer was evaluated in a study of 121 cases and 226 control subjects within the α-Tocopherol, β-Carotene Cancer Prevention Study of male Finnish smokers. Levels of IgG antibodies to *H. pylori* whole-cell and $CagA^+$ antigens from stored baseline serum were measured by enzyme-linked immunosorbent assay. Seroprevalence of *H. pylori* was 82 and 73% among case and control subjects, respectively. Compared with seronegative subjects, those with *H. pylori* or $CagA^+$ strains had a statistically significantly elevated risk of pancreatic cancer, suggesting a possible role for *H. pylori* carriage in development of exocrine pancreatic cancer.

Ovarian Cancer Among *BRCA1* or *BRCA2* Carriers

The effects of parity and oral contraceptive use on the risk of ovarian cancer among *BRCA1* and *BRCA2* carriers and noncarriers were estimated in a case-control study among Israeli women. Of the control subjects who underwent mutation analysis, 1.7% had a *BRCA1* or *BRCA2* mutation, whereas 29% of women with ovarian cancer had a *BRCA1* or *BRCA2* mutation. Overall, each additional birth and each additional year of oral contraceptive use was found to lower the risk of ovarian cancer, as expected. Additional births were protective in separate analyses of carriers and noncarriers, but oral contraceptive use appeared to reduce the risk only in noncarriers. The risk of ovarian cancer among carriers of a *BRCA1* or *BRCA2* mutation decreases with each birth but not with increased duration of oral contraceptive use. These data suggest that use of oral contraceptives for the chemoprevention of ovarian cancer in carriers of such mutations would be premature.

In Utero Exposure to Diethylstilbestrol (DES) in Men

A total of 3,613 men exposed to DES prenatally were studied from 1978 through 1994. Overall, cancer rates among DES-exposed men were similar to those of unexposed men and to national rates for all men. However, the risk for testicular cancer among exposed men was 3.1 times that among unexposed men and 2.0 times the population-based rate.

Pesticides and Non-Hodgkin Lymphoma (NHL)

Data from three population-based case-control studies conducted in Kansas, Nebraska, Iowa, and Minnesota were pooled to evaluate the relationship between pesticide use and NHL among white male farmers. Use of organophosphate pesticides was associated with a statistically significant 50% increase in NHL risk, but direct interviews showed a significantly lower risk (OR = 1.2) than proxy interviews (OR = 3.0). Direct interviews indicate that risk of small lymphocytic lymphoma increases with diazinon use

(OR = 2.8) after adjustment for other pesticide exposures.

In a second study, farmers who had ever used carbamate pesticides had a 30–50% increase in NHL risk, whereas farmers who had not used carbamate pesticides showed no increased risk. Analyses for individual carbamate pesticides found a more consistent association with Sevin than with carbofuran, butylate, or *S*-ethyl dipropylthiocarbamate plus protectant. Among farmers using Sevin, NHL risk was limited to those who personally handled the product, first used the product at least 20 years before their disease diagnosis, or used the product for a longer period. These associations persisted after adjustment for other major classes of pesticides.

Childhood Leukemia and Exposure to Household Solvents

This study explored the risk of acute lymphoblastic leukemia during childhood associated with participation in hobbies or other home projects involving organic solvents. Leukemia was associated with frequent (more than 4 times per month) exposure to model building and with artwork involving solvents. Children whose mothers lived in homes painted extensively (more than four rooms) in the year before the children's birth also exhibited elevated risk.

T-Cell Lymphomas in AIDS

The risk of T-cell lymphoma in the 2 years after AIDS onset was examined by linking data from 302,834 adults with AIDS to cancer registry data. Of 6,788 cases of NHL with specified histologies, 1.4% were T-cell lymphomas. The relative risk of T-cell lymphoma, estimated by the standardized incidence ratio, was 15.0%. Risk increased for all subtypes, including mycosis fungoides, peripheral or cutaneous lymphomas, and adult T-cell leukemia or lymphoma. Nevertheless, although HIV-related immunodeficiency may be an important risk factor, being an immigrant from the Caribbean region and other factors might independently increase the risk for T-cell lymphoma.

Detection, Diagnosis, and Prognosis

Promotion of research to improve cancer screening and early cancer detection and to develop more accurate diagnostic techniques is critical to NCI. NCI-supported research conducted at multiple centers throughout the United States and by intramural scientists is leading to rapid advances in these areas.

Human Papillomavirus (HPV) Testing for Pap Abnormalities

Testing for HPV presence may improve decisionmaking by doctors for their patients with the mildly abnormal and very common Pap test result ASCUS (atypical squamous cells of undetermined significance). Findings from a randomized multicenter study, the ASCUS/LSIL (low-grade squamous intraepithelial lesion) Triage Study (ALTS), by NCI researchers show that HPV testing is highly sensitive in detecting the underlying abnormalities that require immediate attention. Three strategies were compared in the trial: immediate colposcopy; Pap tests repeated every 6 months, followed by colposcopy if more severe abnormalities occurred; and HPV triage in which Pap specimens were tested for HPV types associated with cervical cancer. The HPV testing identified virtually all (96.3%) of the ASCUS abnormalities that needed treatment. Data from the ALTS trial and other cervical screening studies will be evaluated by medical groups at two conferences to develop evidence-based guidelines for clinical practice.

Screening for Ovarian Cancer

NCI intramural researchers in partnership with intramural investigators from the Center for Biologics Evaluation and Research and the Food and Drug Administration (CBER/FDA) have used advanced proteomic analysis to discover a combination of serum proteins (a protein signature) that is diagnostic for ovarian cancer. This signature was derived by identifying the protein differences between the serum protein profiles from unaffected women and from women with ovarian cancer. The advantage of this technique is that the relevant proteins only need to be quantitated and not identified. The signature obtained was evaluated with 116 blinded serum

samples and found to have a positive predictive value of 94%, compared to 34% for CA125, currently the best marker for ovarian cancer.

Chromosome 1 Abnormality and PCV Therapy for Oligodendroglioma

Gliomas are malignant tumors of the brain that are usually fatal within a short time despite treatment. However, one major subtype of glioma, oligodendroglioma, often responds to therapy. Long-term remissions are seen in approximately two-thirds of patients with oligodendrogliomas treated with a combination of procarbazine, lomustine (CCNU), and vincristine (the PCV regimen). Until recently, identifying these patients in advance was not possible. NCI-supported researchers at Massachusetts General Hospital, collaborating with investigators in Ontario, Canada, have discovered that loss of a portion of chromosome 1 in oligodendroglioma tumor cells predicts response to PCV; this finding has been confirmed by a second group of NCI-funded investigators based at the Mayo Clinic. Tumors in these distinct prognostic categories cannot be distinguished on the basis of their appearance under the microscope. Active studies are under way to identify the genes on those portions of chromosomes 1 and 19 that are lost, and these discoveries should improve physicians' ability to recommend appropriate treatments for patients.

Cancer Treatment

NCI supported research leading to numerous treatment-related advances in 2001.

Thalidomide/Immunomodulatory Drugs (Thal/IMiDs), Proteasome Inhibitor (PS-341), and Arsenic Trioxide Treatment for Multiple Myeloma

Thal/IMiDs, proteasome inhibitor (PS-341), and arsenic trioxide are directed at targets in the multiple myeloma cell and its microenvironment. They inhibit tumor necrosis factor α (TNFα)-induced upregulation of adhesion molecules on both multiple myeloma cells and bone marrow stromal cells, as well as related binding; cytokine (interleukin-6 [IL-6], VEGF) transcription and secretion in bone marrow stromal cells triggered both by multiple myeloma cell adhesion and by cytokines (TNFα); and the protective effect of cytokines (insulinlike growth factor [IGF]-1) against multiple myeloma cell apoptosis. Fifteen of 19 patients with multiple myeloma refractory to high-dose therapy and Thal either achieved stable disease or responded to escalating doses of IMiD CC5013 with no side effects other than leukopenia. Seventy-five patients with refractory relapsed multiple myeloma have been treated with PS-341 in a phase II study; 50% responded to treatment, and 33% achieved stable disease. A 23% response rate has been reported in a small phase II study of arsenic trioxide alone in heavily pretreated refractory myeloma patients. Similar results have been reported for a phase I trial combining arsenic trioxide with ascorbic acid.

Gleevec (STI 571) and Gastrointestinal Stromal Tumors (GISTs)

GISTs, mesenchymal neoplasms derived from the interstitial cells of Cajal, are characterized by expression of the protooncogene *c-kit* and often harbor *KIT* mutations leading to ligand-independent kinase activations. GISTS are unresponsive to standard sarcoma chemotherapy. STI571 is an orally available small molecule inhibitor of *Abl, KIT,* and platelet-derived growth factor receptor. Impressive clinical activity has been observed in GIST with STI571 in an EORTC phase I study and a phase II study sponsored by Novartis. STI571 was approved for GIST by the FDA on a fast-track basis.

Proposed Treatment for AIDS-Associated Primary Effusion Lymphoma (PEL)

PEL is a subset of NHL that is detected in the setting of severe immunodeficiency and AIDS. PEL differs from most AIDS lymphomas by the absence of a discernable tumor mass. PEL is always associated with human herpesvirus-8 (HHV-8), whereas other AIDS-related NHL may be associated with Epstein-Barr virus (EBV). In general, lymphomas in the HIV setting tend to be aggressive with poor outcomes. Investigators at the University of Miami have discovered that azidothymidine (AZT) induces apoptosis in EBV^+ NHL but requires addition of interferon-α (IFN-α) to induce apoptosis in

HHV-8^+ PEL cell lines. The mechanism of IFN-α synergy with AZT involved IFN-α-specific induction of high levels of the death receptor TNF-related apoptosis-inducing ligand (TRAIL). IFN-α did not induce TRAIL in EBV^+ or EBV^- primary NHL lines. A marked clinical improvement was observed in an HIV^+ PEL patient who had lymphomatous meningitis treated with parenteral AZT and IFN-α and failed to respond to conventional chemotherapy. This may be a targeted antiviral approach to treating PEL that is effective and nontoxic.

Childhood Acute Lymphoblastic Leukemia (ALL) and Dana-Farber ALL Consortium Protocol 87-01

The purpose of this study was to improve efficacy and reduce toxicity of treatment for children with ALL. Patients from all risk groups, including infants, were treated between 1987 and 1991. At a median followup of 9.2 years, the 9-year event-free survival was about 75% for all patients. The outcome for children with ALL has steadily improved during the past two decades, resulting in an increased cure rate. Despite these advances, two challenging problems remain: how to further increase the cure rate for the 20–25% of children who are not cured by current regimens and how to minimize long-term sequelae for children who are cured.

Doxorubicin and Cardiotoxicity and Dana-Farber ALL Protocol 91-01

Acute doxorubicin-induced cardiotoxicity can be prevented in adults by continuous infusion of the drug, but mechanisms of cardiotoxicity are different in children. Continuous doxorubicin infusion over 48 hours for childhood leukemia did not offer a cardioprotective advantage over bolus infusion. Both regimens were associated with progressive subclinical cardiotoxicity. Other cardioprotective strategies should be explored.

Research Initiatives

Funded Activities

- Interdisciplinary teams for molecular target assessment were funded to focus on a critical biological process that is thought to contain high-priority targets for cancer drug discovery.
- The American College of Radiology Imaging Network (ACRIN), NCI's cooperative group for imaging studies, initiated numerous clinical trials on rare cancers, including
  - Role of Radiology in the Pretreatment Evaluation of Invasive Cervical Cancer
  - Whole-Body MRI in the Evaluation of Pediatric Malignancies
  - Radiofrequency Thermal Ablation of Malignant Hepatic Tumors: A Controlled Clinical Trial To Determine Complete Kill Rates of Ablated Tumors
  - Gleevec for Primary and Recurrent Operable Malignant Gastrointestinal Stromal Tumor (GIST) in Collaboration With RTOG
- The Rapid Access to Prevention Intervention Development (RAPID) program uses the Institute's contract resources to help academic investigators expedite preclinical and early clinical drug development of investigational agents with the potential to prevent, reverse, or delay carcinogenesis. A list of current RAPID projects is available at www3.cancer.gov/prevention/rapid/projects.html. Through a contract or grant mechanism, NCI is also conducting clinical trials of promising prevention agents to develop pharmacokinetic, safety, and toxicity profiles (phase I trials), to demonstrate preliminary efficacy and to develop biochemical and biological surrogate markers of efficacy (phase II trials). These key studies are the stepping stones to large-scale clinical trials in which the value of prevention agents will be determined (phase III trials). Currently, 12 phase I trials and more than 25 phase II and III trials are directed at orphan diseases that are either active or approved.
- NCI continues to screen new synthetic and natural compounds for antitumor activity using the automated cancer cell line screen. Approximately 79,000 defined chemical structures have been evaluated since the screen became operational in April 1990.

More than 7,500 compounds have demonstrated in vitro antitumor activity, of which more than 4,000 agents have been selected for in vivo evaluation for assessment of therapeutic activity. Obviously, there are more compounds to test/develop than current resources would allow. The Drug Development Group (DDG) oversees the decisionmaking process regarding the development of new drugs and relies on extramural review of proposed activities. A complete description of this process is available on the Developmental Therapeutics Program Web site (dtp.nci.nih.gov). Including vaccines and other biologicals, as well as chemotherapeutic agents, 33 agents are in DDG level 1B (early preclinical testing), 14 are in DDG level 2A (GMP production and late preclinical testing), 1 agent is in DDG level 2B (IND-directed toxicology), and 22 are in DDG level 3 (ready for human testing subject to obtaining an IND). Table 1 lists the agents in the DDG process. As the agents move through the different levels of the decision process, the level of NCI's financial commitment increases.

- To expedite the movement of academic discoveries from the laboratory to proof of principle clinical trials, NCI initiated the program Rapid Access to Intervention Development (RAID) in 1998. RAID makes resources available, on a competitive basis, to the academic research community that are necessary to convert a new molecule into a drug candidate suitable for clinical testing and are generally not available to academic investigators who lack a corporate partner. These resources include 1) GMP synthesis, formulation, range finding, and IND-directed toxicology and pharmacology; 2) clinical trial planning; and 3) regulatory assistance so that FDA requirements may be satisfied by any investigator who seeks to put a new molecule into the clinic. As of April 2002, 194 applications have been received, 62 of which were approved for NCI support, and 15 of which are awaiting review. A description of the successful applicants and the projects can be found at dtp.nci.nih.gov/docs/raid/raid_index.html.

  Table 4 lists RAID projects pertaining to rare diseases. Of 62 approved RAID projects, 25 include development of agents for rare disease indications, 5 of which will enter clinical trials by the end of 2002.

- NCI has begun a study to examine whether the prevalence of the C677T and A1298C polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) gene differs between pancreatic cancer patients and control subjects. The MTHFR enzyme is critical in the regulation of folate and methionine metabolism. Folate is required for biosynthesis of oligonucleotides needed for DNA repair mechanisms. Inadequate folate has been associated with human cancer, and recent evidence suggests that folate may play a role in cancer prevention. Analyses will provide new and provocative data for pancreatic cancer.

- NCI-supported investigators have begun a study to identify a comprehensive cardiac risk factor profile on all eligible long-term survivors of childhood cancer within upstate New York. The investigators will determine whether there are increased risk factors and whether these risk factors are related to prior oncologic therapy. This study should enable more rational recommendations for preventive cardiology in long-term survivors to be made and to standardize care and management for this population.

New Initiatives

- Barrett's Esophagus, Gastroesophageal Reflux Disease, and Adenocarcinoma of the Esophagus, a joint request for applications (RFA) issued by NCI and NIDDK, is designed to stimulate and solicit studies to broadly address the problem of Barrett's esophagus, its etiology and relation to gastroesophageal reflux disease, and its link to the rising incidence of adenocarcinoma of the esophagus.

- The RFA Cooperative Grants for Nutritional Modulation of Genetic Pathways Leading to Cancer will create cooperative specialized centers for both basic and clinical research in areas related to dietary nutrients as modi

fiers of genetic pathways leading to cancer. Examples of areas of research are carcinogen bioactivation, cell-cycle control, and signal transduction; intercellular communication and apoptosis; and immune effectors and angiogenesis.

Cosponsorship of Workshops

In 2001, NCI cosponsored eight workshops with ORD. These workshops focused on

- Collaborative Chornobyl thyroid research projects.
- Estimation of thyroid doses resulting from atmospheric nuclear weapons tests.
- Studies on the etiology of brain cancer.
- Leukemia in Chornobyl cleanup workers.
- Lymphomatoid granulomatosis: pathogenesis, pathobiology, and treatment.
- Molecular pathogenesis of human hepatocellular carcinoma.
- Nutrigenomics and nutriproteomics in human disease prevention.
- The pediatric long-term followup clinic and program.

TABLE 1: Compounds That Passed Drug Development Group (as of April 1, 2002)

Drug Development Group 1B	Drug Development Group 2A
NSC Number	**NSC Number**
281617 Dimethane sulfonate	684682 Saporin immunoconjugate: BU12-saporin
281817 Dimethane sulfonate	684683 Saporin immunoconjugate: OKT10-saporin
696823 Discreet	684684 Saporin immunoconjugate: 4KB128-saporin
696824 Discreet	710305 Discreet
696825 Discreet	701315 Anti-HER2 immunoliposomes
696826 Discreet	703939 RFB4-onconase
680410 Adaphostin	709399 Synerlip-p53
718329 Discreet	694501 Discreet
656240 Dithiophene and derivatives	713205 Halofuginone
682994 Dithiophene and derivatives	707016 Discreet
719664 *2-Methoxy antimycin	716976 BNP7787
719239 Discreet	719664 2-Methoxy antimycin A3
707545 17-DMAG	722302 Discreet
720811 Discreet	722333 Transferrin-doxorubicin conjugate
722596 Discreet	644800 IL-7
309132 Zebularine	
711193 Discreet	**Drug Development Group 2B**
713200 Discreet	**NSC Number**
722134 Discreet	710464 Aminoflavone
722135 Discreet	
722136 Discreet	**Drug Development Group 3**
722139 Discreet	**NSC Number**
722142 Discreet	700553 Discreet
722144 Discreet	603573 HeFi-1 anti-CD30 monoclonal antibody
702825 Discreet	710084 Discreet
716239 Discreet	710427 Discreet
716240 Discreet	713219 SGN-00101 (Hsp-E7)
723263 Discreet	702827 SU6668
713713 Discreet	713763 BMS-275291
713719 Discreet	714373 LY353381-HC1
713721 Discreet	659853 2-Methoxyestradiol
722207 Discreet	715969 CAMPATH-1H
720735 Discreet	716711 Epratuzumab (hLL2)
	716976 BNP7787
	698215 R(+)XK469
	678516 ^{18}F-FMAU
	718781 OSI-774
	719850 MEDI-522
	683864 Rapamycin analog
	639829 Dimethyl benzoylphenylurea
	716051 STI571
	706995 MS-275
	710085 IDEC-Y2B8 radiolabeled anti-CD20 antibody
	715055 ZD1839

Table 2: Active Research and Development Agreements, by Agent, Company, and Type (as of April 2002)

Agent	Company	Type
2-Methoxyestradiol	EntreMed	CRADA
280-446	Novartis	CTA
5-Azacytidine	Pharmion	CTA
506U78	GlaxoSmithKline	CTA
AE-941 (shark cartilage)	Aeterna	CTA
All-trans retinoic acid	Roche	CTA
Antigen genes formulated for delivery in a dermal powderject XR gene delivery device	PowderJect	CTA
Arsenic trioxide	Cell Therapeutics	CRADA
Benefin	LaneLabs-USA	CTA
BMS 214662 (FTI)	Bristol-Myers Squibb	CTA
BMS 247550 (epothilone B analog)	Bristol-Myers Squibb	CTA
BMS 275291 (MMPI)	Bristol-Myers Squibb	CTA
BNP7787	Bristol-Myers Squibb	M-CRADA
CCI-779	BioNumerik	CTA
CD40L	Wyeth-Ayerst Research	CRADA
Clodronate	Immunex	M-CRADA
COL-3	Anthra	M-CRADA
CpG ODN	CollaGenex	CRADA
CTLA4-IG	Coley Pharmaceutical Group	CSA
Decitabine	RepliGen	CTA
Depsipeptide (FR901228)	SuperGen	CRADA
Doxil	Fujisawa	CTA
E7389	ALZA	CSA
EMD 121974	Eisai Research Institute	CRADA
Endostatin	Merck KGaA	CRADA
Exemestane	EntreMed	CRADA
Flavopiridol	Pharmacia	M-CRADA
G3139	Aventis	CRADA
Gadolinium texaphyrin	Genta	CRADA
GM-CSF	Pharmacyclics	CRADA
Herceptin	Immunex	CTA
Homoharringtonine	Genentech	CRADA
HSP-E7 (SGN-00101)	American Bioscience Stressgen Biotechnologies	CRADA
HUM291 (anti-CD AB)	Protein Design Labs	CTA
IL-12	Genetics Institute	CTA
IL-2	Chiron	CRADA
		CTA
Iododoxorubicin	Pharmacia	CTA
Iressa (ZD1839)	AstraZeneca	CTA
Irinotecan	Pharmacia	CTA
Lutetium texaphyrin	Pharmacyclics	CRADA
MAGE-3 peptide vaccine	GlaxoSmithKline	CTA

Agent	Company	Type
MGI 114	MGI Pharma	CTA
MS-275	Nihon Schering KK	CRADA
MTP-PE	Jenner Technologies	CTA
O6-BG	AOI	CRADA
ONYX-015	Onyx	CRADA
OSI-774	OSI	CTA
Oxaliplatin	Sanofi-Synthelabo	CRADA
P53 adenovirus	Aventis	CRADA
Perifosine (D-21266)	Zentaris AG	CRADA
PS-341	Millennium	CRADA
PSC-833	Novartis	CTA
PV701	Pro-Virus	CRADA
QS-21	Aquila	CSA
R115777	Johnson & Johnson Pharmaceutical R&D	CTA
Rebeccamycin analog	Exelixis	CTA
rF-TRICOM, rF-CEA-TRICOM	Therion	Intramural
rhuMAb VEGF (Bevacizumab)	Genentech	CRADA
Rituximab	IDEC Therion	CTA
rV-B7.1	Searle	CRADA
SC-55494	Protein Design LabsNovartis	ICRADA
SMART 1D10 (Hu1D10)	Sugen	CTA
STI571	SugenBristol-Myers Squibb	CTA
SU5416	Aventis	CRADA
SU6668	Celgene	CTA
Taxol	Sanofi-Synthelabo	CTA
Taxotere	GlaxoSmithKline	CTA
Thalidomide	Boehringer Ingelheim	CTA
Tirapazamine	Kyowa Hakko Kogyo	CTA
Topotecan hydrochloride	Bristol-Myers Squibb	CTA
Tumor necrosis factor-α	IDEC	CTA
UCN-01		CTA
XK469		CTA
Zevalin (Y2B8)		CTA

Table 3: Investigational New Anticancer Agents in Early Clinical (as of April 2002)

Phase I	Phase II
Cytotoxic Agents	
17-AAG	Arsenic trioxide
2-ME	BMS 247550 (epothilone B)
Arsenic trioxide	BMS 275291 (MMPI)
BMS 214662 (FTI)	Bryostatin 1
BMS 247550 (epothilone B)	BSO
BMS 275291 (MMPI)	CAI
BPU	CI-980
CCI779	Chloroquinoxaline sulfonamide (CQS)
COL-3	COL-3
Compound 506U78	Compound 506U78
Depsipeptide	Depsipeptide
E7389 (halichondrin B analog)	Dolastatin 10
EF5	Fenretinide
EMD 121974	Flavopiridol
Flavopiridol	G3139
G3139	Iododoxorubicin
Iododoxorubicin	Iressa
Irofulven (MGI-114)	Irinotecan (CPT-11)
KRN5500	Irofulven (MGI-114)
Lutetium texaphyrin	O_6-BG
MS-275	OSI-774
O_6-BG	Phenylacetate
Perifosine	PS341
Phenylbutyrate	PSC 833
PS341	Pyrazoloacridine
PSC 833	Pyrazine diazohydroxide
Rebeccamycin analog	R115777
SarCNU	Rebeccamycin analog
STI571	SarCNU
SU5416	STI571
SU6668	SU5416
Tirapazamine	Tirapazamine
UCN-01	Topotecan
XK469	
Biological Agents	
Adeno-p53 (Advexin)	Adeno-p53 (Advexin)
ALVAC-B7.1/StemSep	Anti-idiotype-KLH lymphoma vaccine
Anti-idiotype-KLH myeloma vaccine	Avastin (bevacizumab, MoAb: Anti-VEGF)
Anti-Tac (Fv)-PE38 immunotoxin	Carboxypeptidase G2
Avastin (bevacizumab, MoAb: anti-VEGF)	D1/3 MAGE
BL22 immunotoxin	ESO-1 peptide
Carcinoembryonic antigen peptide vaccine	Fowlpox-PSA vaccine
CEA vaccinia vaccine	Fowlpox-gp100:ES209-217(210M) vaccine
Endostatin	
FLT3 ligand	

Phase I	Phase II
Fowlpox-gp100 vaccine gp100 melanoma vaccines HeFi-1 Herceptin (trastuzumab; MoAb: humanized Her2) HER-2/neu peptide vaccine HPV E6 & E7 vaccine HPV E7 lipopeptide vaccine HuID10 HuM291 IL-12 IL-12 + IL-2 Immunotoxin ERB-38 LMB-2 LMB-9 MAGE-12 peptide vaccine MART-1 melanoma vaccines MDX-010 MEDI 522 MoAb: B3 MoAb: 14.18 chimeric MoAb: CC49-9OY MoAb: T cell (3A1, 95-5-49, 95-6-22) MOV-18 chimeric T-cell receptor ONYX-015 (E1B-attenuated adenovirus) P53 and RAS peptide vaccine Pediatric sarcoma peptide vaccines pg100 IVS cells vaccine PR-1 peptide PV701 rF-GM-CSF SGN-00101 Vaccinia B7.1 + fowlpox B7.1 Vaccinia-CEA-TRICOM + fowlpox-CEA-TRICOM Vaccinia-gp100 vaccine Vaccinia-MUC1 vaccine Vaccinia-TRICOM + fowlpox-TRICOM VHL peptide vaccine Zevalin (Y2B8)	IFN: Rec-γHerceptin (trastuzumab; MoAb: humanized Her2) HPV E6 & E7 vaccine gp100 DNA gp100 melanoma vaccines IL-21TIL MART-1 melanoma vaccines P53 and RAS peptide vaccine PG13/LNc8 transduced T cells Rituxan (Rituximab, MoAb: C2B8) SGN-00101 Telomerase:540-548 peptide Vaccinia tyrosinase + fowlpox tyrosinase

Table 4: RAID Compounds for Treatment of Rare Diseases (as of April 2002)

Compound NSC	Name	Disease	Investigator	Pediatric Use
710296	C-myb antisense oligodeoxynucleotide	Acute myelocytic leukemia	Alan Gewirtz University of Pennsylvania, School of Medicine	Yes
710292	Lipopeptide	Cytomegalovirus	Don Diamond City of Hope Medical Center	Yes
354258	8-Chloro-adenosine	Multiple myeloma	Steven Rosen Northwestern University, Lurie Comprehensive Cancer Center	
711516	Chimerized antiamyloidosis Moabs	AL amyloidosis	Alan Solomon University of Tennessee	
711517	Shed polyvalent antigen vaccine	Melanoma	Jean-Claude Bystryn New York University Medical Center	
711295	Moab 216 (VH4-34) Anti-Hu B lymphocyte antibody	Lymphoma	Nelson N.H. Teng Stanford University	Yes
711518	Allogenic pancreatic tumor vaccine	Pancreas	Elizabeth M. Jaffee Johns Hopkins University	
711519	IGF-1R antisense oligodeoxynucleotide	Glioma	Robert Aiken Thomas Jefferson Medical College	Yes
714597	Imexon	Multiple myeloma	Robert Dorr University of Arizona, Arizona Cancer Center	
113090	Betulinic acid	Multiple myeloma	Tapas Das Gupta University of Illinois at Chicago	
650378D	Spongistatin 1	Melanoma, ovary	George Pettit Arizona State University, Cancer Research Institute	
734551 714503	Fenretinide plus safingnol	Neuroblastoma, pancreas, acute leukemias	C. Patrick Reynolds University of Southern California School of Medicine	Yes
715815	Chimeric anti-CD54 Moab (UV3)	Multiple myeloma	Ellen Vitetta University of Texas, Southwestern Medical Center	
715816	Tropism-modified adenoviral vector	Ovary	Glenn Peters University of Alabama, Comprehensive Cancer Center	
717904	Immucillin-H	T-cell lymphoma	Vern Schramm Albert Einstein College of Medicine	
7365	6-Diazo-5-oxo-L-norleucine	Neuroendocrine	Håkan Örlefors Uppsala University Hospital, Sweden	
71887	Pseudomonas exotoxin construct	Glioblastoma multiforme, neoplastic meningitis	Darrell Bigner Duke University, Comprehensive Cancer Center	Yes

Compound NSC	Name	Disease	Investigator	Pediatric Use
719277	Nonpathogenic oncolytic poliovirus chimeras	Glioma	Matthias Gromeier Duke University Medical Center	Yes
720454	vac-mTag recombinant Vaccinia construct	Mesothelioma	Harvey Pass Barbara Ann Karmanos Cancer Institute	
720833	EBV supernatant	Lymphoproliferative disease	Richard Ambinder Johns Hopkins University	
720836	IL-6 plus IFN	Multiple myeloma	Richard Jones Johns Hopkins University	
722667	Folate receptor-targeted liposomal daunorubicin (F-L-DAU)	Acute myelocytic leukemia	Robert Lee Ohio State University	Yes
722756D 722757D	5H4 and 8B9	Lymphoma	Benjamin Rich Brigham and Women's Hospital	
723253	Allogeneic multiple myeloma vaccine	Multiple myeloma	Ivan Borrello Johns Hopkins University	
723256	δ-24-RGD oncolytic virus	Chronic lymphocytic leukemia	Alfred Yung University of Texas, M.D. Anderson Cancer Center	

National Institute of Child Health and Human Development (NICHD)

Overview

NICHD's mission is to conduct and support research on the physiological and behavioral processes that determine the health of individuals and populations. The Institute's programs are based on the concept that adult health and well-being are partly determined by episodes early in life and that human development continues throughout the lifespan. Diseases or conditions that interfere with healthy development are of concern to the Institute; thus, NICHD supports research in prevention, diagnosis, evaluation, and treatment of many rare diseases and disorders.

Recent Scientific Advances

Familial Incontinentia Pigmenti (IP2) and Dyskeratosis Congenita (DKC)

IP2 is an inherited disorder affecting the skin and its derivatives, the eyes, and the central nervous system. The gene for this condition is carried on the X chromosome. As a dominant trait, females need inherit only one gene to display features of the disorder; males who inherit this gene usually die before birth. IP2 is diagnosed at or soon after birth by the presence of a progressive redness of the skin and a rash with small fluid-containing bumps. Over time, the skin becomes rough, warty, and pigmented. By adolescence, pigmentation is lost in some areas, leaving a lined or netlike pattern on the skin. Other manifestations include absence of hair, absence of one or more teeth, defective nails, neurological complications such as seizures, and vessel abnormalities of the retina.

Molecular and genetic approaches are being used to identify candidate genes for IP2 by refining and pinpointing the IP2-critical region on the X chromosome. Genomic sequence data are analyzed as they become available. With the aid of IP2 patients and their families and mouse models of the disorder, the complete sequence and genomic organization of the *IP2* gene and the subcellular location of protein made by the gene are being determined. DKC is similar to IP2, and patients share many of the same physical defects. The *DKC* gene is also carried on the X chromosome. Recent studies have determined that, although the genes for these disorders are located in the same small region of the X chromosome, variations of the *IP2* gene do not cause DKC—they are two distinct genes.

Severe Combined Immunodeficiency Syndrome

NICHD, in collaboration with NIAID, NCI, and the Jeffrey Modell Foundation (JMF), continues to support and conduct basic, clinical, translational, and epidemiological research on inherited or primary immunodeficiency diseases in children. Moreover, NICHD sponsors educational and outreach activities to enhance the awareness of primary immunodeficiency diseases among the general public, primary-care practitioners, and pediatricians. For the past year, the NICHD/JMF booklet *Primary Immunodeficiency: When The Body's Defenses Are Missing*, has been distributed nationally to investigators, primary-care physicians, health-care professionals, and the general public. The booklet is especially informative and valuable to immunodeficient patients and their families. It highlights the 10 warning signs for primary immunodeficiency and is a valuable resource for professional assistance, treatment, and patient support groups. To expand readership and accessibility, the entire booklet was added to the NICHD Web site, with links to the NIAID and the JMF Web sites.

NICHD, NIAID, and NCI also continue to jointly support an epidemiological research project to determine the prevalence of primary immunodeficiencies in minority and white populations in New York City. The primary aims of the project are to examine whether there is a difference in prevalence of primary immunodeficiencies in the two populations and, if so, to ascertain the reasons for the differences. The project

is also designed to educate physicians and health-care workers who care for minorities about primary immunodeficiencies. During the past year, the same three Institutes have held joint meetings and workshops to explore new opportunities and challenges for research in primary immunodeficiencies: Advances in the Diagnosis and Treatment of Primary Immunodeficiency Diseases: Risk of Cancer; Gene Therapy for Primary Immunodeficiency Diseases; The Developing Immune System: Frontiers of Knowledge; and Primary Immunodeficiency Diseases Advisory Group Meeting. The meetings were highly productive and generated exciting new ideas, recommendations, and innovative proposals for future NIH research initiatives in primary immunodeficiencies.

Infantile Neuronal Ceroid Lipofuscinosis (INCL)

INCL is a rare genetic disorder of the nervous system characterized by progressive motor and cognitive decline, seizures, and visual loss from pigmentation buildup in the retina. These impairments are linked to an abnormal accumulation of ceroid fatty-protein pigments in the body's cells resulting from a deficiency of a specific enzyme that normally breaks up these compounds. Various drugs can break the chemical bonds that are specifically involved in accumulation of the destructive protein complex. Several drugs were tested, and phosphocysteamine, a drug used in the treatment of cystinosis, was selected for further characterization. In cultured cells from INCL patients, phosphocysteamine directed the breakdown of ceroid deposits within the cells and prevented their reaccumulation. This drug also shows promise because it can cross the blood-brain barrier, is nontoxic, and prevents the death of precursors to INCL white blood cells. The cumulative results suggest that the drug might be useful as an effective treatment for INCL. INCL is a uniformly fatal disease for which no effective treatment currently exists. Because the active compound of phosphocysteamine has been in clinical use safely for more than two decades, NICHD is testing the effects of the Food and Drug Administration-approved version of this drug, cysteamine bitartrate, on five patients in a pilot study. If therapy provides clinical stabilization or improvement in these patients, the investigation may be extended to a controlled multicenter study.

Prader-Willi Syndrome (PWS)

PWS is characterized by many complex physical and behavioral findings, including delayed psychological and motor development, mental retardation, short stature, underdeveloped reproductive organs, overeating, moderate to severe obesity, and behavioral peculiarities. More than half of PWS patients have abnormalities on chromosome 15. PWS is specifically associated with food-related behaviors characterized by out-of-control eating, food foraging and hoarding, decreased perception of feeding satisfaction, and obsessions about food. Although less well described, many people with PWS show obsessions and compulsions not related to food, as well as significant behavioral and emotional dysfunction.

The bulk of work centered on PWS is concentrated on the unusual genetics of the disorder, leaving a void in behavioral research. Although NICHD is funding genetic research, it is also focusing on behavioral research specific to PWS by attempting to identify and characterize mechanisms of compulsivity and other maladaptive behaviors. Ongoing investigations are examining how obsessive-compulsive tendencies in people with PWS contribute to their difficulties in learning and food-related behaviors. Investigations are seeking to determine how the consistency of food-associated cues interact with obsessive-compulsive characteristics to influence learning about food and development of food-seeking behavior. The onset, course, and occurrences of obsessive-compulsive symptoms in PWS are also being compared with those children who have obsessive-compulsive disorder. In addition, mechanisms in the nervous system that underlie food-seeking behavior and learning performances associated with obsessive-compulsive behavior are being explored via functional magnetic resonance imaging of the brain. Together, these studies will help fill the longstanding behavioral research void of this complex developmental disorder.

Animal Models

Down Syndrome (DS)

DS is a chromosomal abnormality caused by the presence of a third whole chromosome 21, usually contributed by the mother. DS individuals are mentally retarded, have decreased muscle tone, and show symptoms of Alzheimer disease after age 35. This syndrome has an estimated prenatal lethality of 70–80%. Recent analysis of human chromosome 21 has shown that it consists of 225 identified genes, making it relatively gene poor. There are several known genes on this chromosome for which excess expression might contribute to symptoms of mental retardation. Few studies have researched the electrical properties of the nerve cells in DS, and none have focused on the hippocampus, the part of the brain responsible for learning and memory. To help circumvent this problem, a mouse model (Ts65Dn) has been developed that has an extra copy of a segment of chromosome 16, which is identical to a segment of human chromosome 21. This segment of chromosome 21 is thought to be responsible for mental retardation and vulnerability to Alzheimer disease. The Ts65Dn mouse survives into adulthood and demonstrates impaired behavior and learning. Neural electrical activity that has been related to learning and memory is impaired in a certain region of the hippocampus of the Ts65Dn mouse. Researchers are testing whether these anomalies are due to abnormal activity of enzymes manufactured by the extra copy of genes on chromosome 16 and whether the abnormalities are connected to learning and memory. Studies with this mouse model may help in understanding mechanisms of mental retardation in DS, identifying specific targets for DS therapy, and providing clues to the chemical activities of the brain involved in learning and memory.

Hemolytic Disease of the Newborn (HDN)

HDN begins when antibodies manufactured by the mother to the Rh protein carried on the red blood cells of the fetus are passed through the placenta to the fetus. These antibodies attack the blood cells of the fetus causing fetal anemia, fetal edema, and even death. Although women have been successfully treated to prevent this condition in their newborns, a few infants show some effect of this disorder at birth. Currently, the intrauterine transfusion of red blood cells to the fetus with ultrasound guidance is the only treatment available for severe hemolytic disease of the fetus. Despite the availability of this life-saving procedure, up to 20% of affected fetuses succumb to HDN. Clearly, an innovative therapeutic approach for mothers must be sought to further improve fetal survival.

An animal model for this condition has been developed with the rabbit. A colony of these rabbits has been established, and the maternal, immunological, fetal, and neonatal aspects of this unique model have been characterized. Immunotherapeutic methods of treating the mother are under way to prevent manufacture of the destructive antibodies. The approach is to obtain female rabbits that were previously exposed to incompatible fetal red blood cells from previous pregnancies and immunize them with multiple paternal blood cell proteins. The aim is to prevent HDN or decrease its severity in subsequent newborns. The mother's antibody response to this treatment will be tested to determine whether it is responsible for any protective effects against HDN development.

Research Initiatives

Osteogenesis Imperfecta

This request for applications (RFA) is intended to stimulate and support new research projects that have the potential to increase understanding of skeletal pathology of osteogenesis imperfecta and lead to improved therapeutic approaches to the disease. In particular, new studies should test the importance of defects in regulation of bone remodeling in the pathogenesis of osteogenesis imperfecta with the view of exploiting pharmacological therapies that may improve the consequences of the underlying genetic defects. A second goal is to increase understanding of the developmental biology of the osteogenesis imperfecta bone precursor cells to form the basis for future therapeutic efforts with genetic modification and transplantation of these cells.

Neural Tube Defects Facility

NICHD reannounced support of the Neural Tube Defects Facility. This facility is a research resource that maintains and distributes mouse models for neural tube defects. It was established more than 10 years ago with the purpose of making these models available to the scientific community at a reasonable cost. Animals are available to all investigators. This facility is supported by contract.

Conferences, Workshops, and Other Activities

A conference titled Prenatal Alcohol Exposure Among American Indians and Alaskan Natives: Relationship to Sudden Infant Death Syndrome was held in August 2001. Certain American Indian and Alaskan Native communities have a high prevalence of maternal drinking during pregnancy. Many of these communities also have high rates of infant mortality, with a significant proportion attributed to sudden infant death syndrome (SIDS); SIDS rates in these areas have about five times the national average. In the Aberdeen Area Indian Health Service Infant Mortality study, a strong association was observed between prenatal alcohol consumption and SIDS. Neurochemical analysis of the myelin lipids and proteins in the brains of the SIDS cases demonstrated an association between decreased myelination and prenatal alcohol exposure, suggesting that damage to the brain occurs early in gestation. The findings are preliminary and must be confirmed. The purpose of this conference was to discuss how these findings can be pursued, along with their relation to the pathogenesis of SIDS and fetal alcohol syndrome (FAS). Participants included members of the American Indian/Alaskan Native communities and experts in the epidemiology, pathology, neurochemistry, and developmental biology of SIDS and FAS.

The Workshop on Chromosome 18: The Current State of the Science was held in July 2001 to bring together basic and clinical researchers familiar with syndromes associated with chromosome 18 and those with expertise relevant to chromosome 18 disorders. The meeting established consensus in the state of research on these disorders and identified critical areas of research where fundamental knowledge relevant to the disorder is lacking. Animal models of relevant conditions associated with mental retardation and developmental disabilities were also discussed. Participants were scientists interested in neuropathology, psychiatry, pediatrics, genetics, neurocognitive development, and learning disorders.

The First Structural Birth Defects Investigator's Meeting was held in July 2001. A primary mission of NICHD is to increase knowledge and understanding of the epidemiology, developmental biology, genetics, and etiology of structural birth defects. Initially, workshops held in 1997 and 1998 resulted in the issuance of two RFAs concerning the underlying mechanisms of birth defects. The current meeting represents an important step in establishing a network of birth defect investigators as recommended by the workshops and the NICHD strategic plan in developmental biology. The 2-day meeting brought together grantees supported by NICHD RFAs on birth defects, providing a forum for discussing their research plans and progress, exchanging ideas, sharing resources, and fostering collaborations to enhance the goals of the NICHD birth defects initiative.

The Warren Grant Magnuson Clinical Center (CC)

Critical Care Medicine Department

Sickle Cell Anemia

Sickle cell anemia is an inherited disease affecting primarily African Americans (0.15% are homozygous for the disease, and 8% have sickle cell trait). Acute pain crisis, acute chest syndrome, and secondary pulmonary hypertension are common complications of the disease. Studies of nitric oxide (a naturally occurring gas) in patients with sickle cell anemia have been a major focus of study in the CC Critical Care Medicine Department over the past 4 years. The investigators have focused on nitric oxide, specifically hemoglobin interactions, transport physiology, and translation to clinical applications. Scientists in the Critical Care Medicine Department, in collaboration with colleagues from NIDDK and NHLBI, have

- Determined the nitric oxide donor properties of hydroxyurea in patients with sickle cell disease and that nitric oxide release from hydroxyurea determines fetal hemoglobin induction by this agent.
- Characterized nitric oxide bioavailability in patients with sickle cell anemia, including developing a better understanding of its binding properties on sickle cell hemoglobin and the nitric oxide scavenging effects of cell-free hemoglobin.
- Collected preliminary data on overall incidence (32%) of pulmonary hypertension in patients with sickle cell anemia via prospectively collected, serial transthoracic echocardiograms. More than 5% of all patients have severe pulmonary hypertension based on echocardiographic criteria. Data also suggest that chronic hemolysis and/or anemia may contribute to the development of secondary pulmonary hypertension.
- Initiated further studies to evaluate chronic nitric oxide inhalation as a therapy for patients with sickle cell anemia and secondary pulmonary hypertension, the addition of nitric oxide donor medications to hydroxyurea to augment fetal hemoglobin induction, and the effects of inhaled nitric oxide gas on vaso-occlusive pain crisis.

Laboratory Medicine Department

Nocardiosis

Nocardiosis is a localized or systemic bacterial infection caused by any one of the dozen or so pathogenic species in the genus *Nocardia*, which are widespread in the environment. Both immunocompromised and immunocompetent individuals may become infected with these organisms; infection is acquired from an environmental source, not from another infected individual. In immunocompromised individuals, infections may become deeply invasive and disseminated; in immunocompetent individuals, infections are usually secondary to trauma and localized. Molecular methods are proving to be the most rapid and reliable techniques for discriminating among the different species in the genus.

The current identification algorithm involves initial amplification of portions of the genes for the heat shock protein and for 16S rRNA, using the polymerase chain reaction, with subsequent cleavage of the amplicons using restriction enzymes; the patterns of the amplicon fragments obtained are compared with the patterns from known organisms to identify an unidentified isolate. When a novel pattern is obtained, the entire *16S rRNA* gene is sequenced in an attempt to identify the unknown organism by comparing its gene sequence with those of known organisms. Use of these procedures has revealed that several pathogenic species have a much broader geographic distribution than previously appreciated; several clinical isolates that probably belong to hitherto undescribed species have also been found. Accurate identification of these organisms is important for defining both their geographic distribution and their relative pathogenicity; rapid identification may facilitate early

selection of optimal therapy for species with different antibiotic susceptibility patterns.

Rehabilitation Medicine Department (RMD)

Juvenile Dermatomyositis

Children with juvenile dermatomyositis present with generalized muscle weakness and decreased joint motion because of lack of strength to move through the entire range. Data collection for this population has been completed, and the data have been analyzed for manuscript preparation. Tests included the manual muscle test, range of motion and a functional ability test, the Pediatric Evaluation of Disabilities Inventory (PEDI), and analyses of gait. RMD reports that weakness of major muscle groups in the lower hip girdle has significant impact on walking speed.

Juvenile Rheumatoid Arthritis

Children with juvenile rheumatoid arthritis (JRA) are referred to RMD for evaluation in two protocols:

1. Children with JRA uveitis (an eye condition affecting many children with JRA) are referred to physical therapy for evaluation of their functional abilities with the PEDI, gait parameters with the stride analyzer, and hand function with the Jebsen hand test.
2. Children with JRA are referred to physical therapy for evaluation of their functional abilities on the PEDI, gait parameters with the stride analyzer, and walking endurance with the 9-minute walk-run test.

RMD's findings in this population indicate that every child evaluated had disability pertaining to the musculoskeletal system, including leg length discrepancies, mechanical abnormalities of foot function requiring orthotics or shoe modifications, and abnormalities of hand prehension and function.

Smith-Magenis Syndrome (SMS)

SMS is a chromosomal disorder (deletion of 17p11.2) characterized by dysmorphic facial features, brachydactyly, short stature, hypotonia, and speech and cognitive delays. Sensorimotor skills, gross motor performance, and gait evaluations by RMD will provide information related to the physical/sensorimotor and behavioral characteristics of children with SMS. RMD staff have evaluated a number of patients to date; findings remain preliminary. Laryngeal pathology, including oral sensorimotor abnormalities such as open-mouth posturing, decreased lingual range, and feeding and swallowing difficulty, occurs in 90% of the population studied.

Congenital Adrenal Hyperplasia (CAH)

CAH denotes a family of inherited disorders with defects in central biosynthesis. CAH has been traditionally considered a disease restricted to the adrenal cortex. Recent findings in animals and in resting adrenaline levels in patients with CAH support the hypothesis that hypofunction of the adrenal medulla may also be a contributing factor. In addition, patients with CAH complain of excessive fatigue, possibly related to low stress catecholamine levels during physical activity. RMD staff are investigating possible differences in exercise responses between adults and children with and without CAH.

McCune-Albright Syndrome (MAS)

Fibrous dysplasia is a benign bone lesion that can affect the entire skeleton. When accompanied by precocious puberty, it is called MAS. Phenotypic expression includes facial asymmetries, joint malalignment, joint laxity, and fractures through the dysplastic bone. RMD staff are investigating the variation in phenotypic expression, gait parameters, and determination of stamina in patients who are 4–59 years of age. RMD staff have determined that those with MAS walk significantly slower than healthy individuals and are likely to benefit from aerobic conditioning programs.

Alkaptonuria

Alkaptonuria is an inherited disorder in which homogentisic acid accumulates deposits in soft tissue and cartilage. RMD staff performed a

thorough musculoskeletal assessment on 58 patients that included measures of range of motion, strength and imaging of muscle, and soft tissue via diagnostic ultrasound. Patients with falkaptonuria have kephalin, loss of truncal flexion, and appendicular joint range, which increases with age (thus, joint replacement is common). Functional assessments (including SF-36 and human activity profile) show a decline with age, but measures of health status (SF-36) pertaining to mental function do not show the same deficits as for physical function.

National Center for Complementary and Alternative Medicine (NCCAM)

Overview

Congress endowed NCCAM with a broad statutory mandate to conduct and support complementary and alternative medicine (CAM) research, support research training, disseminate information, and facilitate integration of CAM and conventional health-care delivery to move the CAM field forward. To fulfill its mandate, NCCAM is undertaking a number of challenges and supporting a broad portfolio of research to expand basic and clinical research, including the prevention, evaluation, and treatment of many rare diseases. The research projects supported by NCCAM also test hypotheses for which minimal preliminary data or lack of a conventional biological rationale exists.

Neurological Disorders

Parkinson Disease (PD)

Three NCCAM-supported studies are focusing on CAM approaches to treat PD. In the first study, investigators are conducting sequential studies of mood and motor function in severely depressed PD patients who have failed antidepressant medication because of adverse effects or lack of efficacy. Depressed PD patients who failed to respond to conventional electroconvulsive therapy (ECT) are first being given the opportunity to receive repetitive transcranial magnetic stimulation (rTMS). Extensive motor, psychiatric, and neuropsychological evaluations are being performed before and after each course of treatment and for 8 weeks after treatment. These investigators are providing an initial estimate for the relative efficacy of rTMS and ECT in PD patients.

In the second study, investigators are comparing two Chinese mind-body modalities (Tai Chi and Qi Gong) as part of a CAM exercise program. Their aim is to examine caloric dose response of exercise treatments on central and peripheral indices of motor control, including electromyographic synchronization of muscle fiber contraction, movement time, choice reaction time, muscle stiffness, global disability, quality of life, mood and anxiety scales, and fitness outcome measures. The third study is comparing two CAM treatments (valerian and melatonin), two conventional therapies, and a placebo control for sleep disorders of PD patients. Outcomes will include both a measure of nocturnal sleep (polysomnographic measurements) and waking motor function to assess improved sleep.

Huntington Disease (HD)

NCCAM is supporting an HD project to assess the dietary supplement creatine as a neuroprotective agent, identify potential mechanisms of creatine neuroprotection in mouse models, and perform phase I and II clinical trials of creatine on early-stage HD patients. Investigators are evaluating whether dietary supplementation with creatine, with and without the metabolic cofactor coenzyme Q10, can delay expression of clinical phenotype, improve survival, and prevent neuronal cell death in animal models of HD. The investigators enrolled 64 early-stage HD patients in a randomized, placebo-controlled, double-blind, open pilot study to assess safety and dosage of daily ingestion of creatine by early-stage HD patients. Outcomes will include measurement of motor skills, laboratory measures of oxidative stress, measures of sensorimotor response, and proxy measures of neuronal degeneration.

Spastic Cerebral Palsy

Two CAM modalities are being evaluated in NCCAM-supported phase II clinical trials: osteopathic manipulation and acupuncture have been used in children to reduce complications associated with cerebral palsy versus a control group (therapeutic play). These clinical trials are further assessing the incorporation of and compliance with the two modalities within the participating groups.

Multiple Sclerosis (MS)

NCCAM is supporting two studies on alternative approaches to MS. The first study is assessing three natural antioxidant regimens as potential treatments for MS: Ginkgo biloba, α-lipoic acid/ essential fatty acids, and vitamin E/selenium. The antioxidant regimens that suppress experimental autoimmune encephalomyelitis are being evaluated for their ability to decrease markers of lipid, protein, and DNA oxidative injury in blood and cerebrospinal fluid in patients with MS. In the second study, a 6-month randomized, controlled phase II trial, investigators are determining whether yoga intervention has a positive effect on measures directly related to yoga practice (flexibility and balance), as well as mood, quality-of-life, and oxidative injury markers.

Cancers

Glioblastoma

Glioblastoma multiforme is the highest grade of malignant glioma, grows rapidly (sometimes doubling in size every 10 days), and is nearly uniformly fatal. NCCAM is supporting two studies on alternative approaches to glioblastoma. In the first study, investigators are examining in vitro the efficacy of berberine, a compound isolated from a Chinese herb, to enhance the radiation sensitivity of glioma cells. In the second study, glioma patients beginning radiation therapy are also enrolled in a double-blind, randomized, controlled study of distant healing intentionality. Distant healing is a mental intention on behalf of one person to benefit another at a distance.

Ovarian Cancer

In a study on alternative approaches to ovarian cancer, NCCAM funded investigators to address the use of acupuncture by traditional Chinese clinicians. The investigators are also assessing the quality of life and symptoms of patients with incurable ovarian cancer. The study populations are women with recurrent metastatic ovarian cancer and similar patients with advanced cancer who are ambulatory and receiving conventional palliative care.

Others Categories

Retinitis Pigmentosa

NCCAM is supporting an exploratory study to assess effects of the dietary supplement lutein on retinitis pigmentosa. The investigators are using standard clinical vision tests that have been developed and adapted for use on a personal computer (PC). The effect of lutein on vision are being studied in a clinical trial comparing placebo with two different dosages of lutein in a randomized, double-blind crossover design. Investigators are testing for possible adverse effects and measuring compliance. Other volunteers were recruited who, along with the first group, monitored their vision every 1–2 weeks at home using the PC-based tests. Results are being validated against those obtained with standard tests during multiple visits to the vision center. The results and tools produced by this study will enable a long-term lutein supplementation trial with vision as its principal outcome measure, and the study can serve as a model for other nutritional supplement trials.

Fibrosis

Curcumin, derived from the spice tumeric and found in Chinese and Indian folk medicine, is used to treat a broad range of autoimmune diseases. NCCAM is supporting research on a curcumin treatment of fibrosis. Patients with scleroderma, a debilitating autoimmune disease, suffer from dermal fibrosis. The hypothesis being tested is that curcumin may be beneficial treatment for scleroderma in particular and fibrotic diseases in general. This research study demonstrates the efficacy and the scientific basis for that efficacy of a disease treatment already recommended by practitioners of alternative medicine.

Lymphedema

Massage therapy, in the form of manual lymph drainage or manual lymphedema (LD) treatment, is an integral component of the international consensus-recommended optimal treatment for LD of the arms and legs. NCCAM is supporting an exploratory study to lay the groundwork and evidence-based rationale for clinical trials with manual lymph drainage alone

and in combination with different forms of mechanical compression in the prevention and treatment of various forms and stages of upper- and lower-limb LD in children and adults.

Temporomandibular Disorder

Two phase II clinical trials are being conducted by NCCAM-funded investigators. The first trial is designed to evaluate select complementary approaches to temporomandibular disorder pain management: acupuncture, chiropractic therapy, and bodywork therapy by clinicians. In the second trial, investigators will study three approaches for addressing patient symptoms: naturopathic medicine, traditional Chinese medicine, and traditional care. The study will evaluate the two alternative healing systems for system relief, safety, acceptability, feasibility, adherence, and improvement in overall quality of life compared with traditional care.

National Institute on Deafness and Other Communication Disorders (NIDCD)

Overview

NIDCD conducts and supports research and research training on normal mechanisms and diseases and disorders of hearing, balance, smell, taste, voice, speech, and language. NIDCD achieves its mission through a wide range of research performed in its own laboratories, a program of research grants, individual and institutional research training awards, career development awards, center grants, cooperative clinical trials, and contracts to public and private research institutions and organizations. The Institute also conducts and supports research and research training related to disease prevention and health promotion. NIDCD addresses special biomedical and behavioral problems associated with people who have communication impairments or disorders and supports efforts to create devices that substitute for lost and impaired sensory and communication functions.

Recent Scientific Advances

Mitochondrial Genes and Deafness

Mitochondria are specialized structures within cells that play a crucial role in metabolism and energy production. Mitochondria contain their own genes, which replicate during cell division. All of the mitochondria in individuals are derived from the mother's egg. Therefore, diseases that appear to be passed exclusively through maternal lineage are often linked to defective mitochondrial genes.

NIDCD-supported scientists have identified several specific mitochondrial mutations that predispose an individual to hearing damage resulting from toxicity to the inner-ear hair cells from the aminoglycoside class of antibiotics. These investigators have determined that genetic loci in the nucleus of the cell act to modify the effects of the mitochondrial mutations. Most recently, a specific gene was identified in mice that modulates the severity of mitochondrial deafness and is also implicated in age-related hearing loss. This mouse model will be extremely valuable for detailed studies of the molecular mechanisms by which mitochondrial mutations contribute to deafness. The findings could be used to develop genetic tests to determine whether an individual has an increased risk for aminoglycoside-induced hearing damage.

Usher Syndrome

Usher syndrome is characterized by hearing loss, retinitis pigmentosa, and absence of vestibular reflexes. This syndrome accounts for about 5% of the deaf population and more than 50% of the deaf and blind individuals (>10,000) in the United States. The severity of the hearing loss and the presence of vestibular dysfunction distinguishes two clinical subtypes of Usher syndrome, 1 and 2. A third form of Usher syndrome (type 3) that has a late onset has recently been described. All three phenotypes involve different genes. NIDCD's intramural research program is continuing to support the Hereditary Hearing Impairment Consortium, the members of which are working to identify and characterize all the genes responsible for Usher syndrome. They have discovered that the gene for Usher type 1B encodes an unconventional myosin protein, and Usher type 1D and 1F genes encode cell adhesion proteins called cadherins. Recently, several NIDCD-supported scientists reported cloning the gene for Usher syndrome type 2A. The *USH2A* gene encodes a protein, usherin, that has structures similar to other proteins involved in assembling cells and tissues into functional organs. NIDCD-supported scientists have identified the genes responsible for Usher type 1C. These advances are critical steps toward developing strategies to treat this devastating disease.

Waardenburg Syndrome (WS)

WS is an autosomal dominant disorder characterized by pigmentary disturbances and deafness. NIDCD-supported scientists seek to determine the loci for WS type 2 by using a high-

density genome scan coupled with linkage analysis to identify candidate gene mutations that could be the cause of this disorder in three large, multigenerational families and several smaller families with WS type 2. Other scientists are studying the Dalmatian as an animal model for understanding the genetics of pigment-associated deafness in dogs and humans. The relationship between pigmentation and deafness is not unique in Dalmatians, a model that offers a unique opportunity to conduct genetic analysis of hereditary deafness.

Auditory Neuropathy

A small but substantial number of individuals with bilateral hearing loss have normal cochlear function. These individuals have severely abnormal central neural processing of auditor sensory input, evidenced by poor or absent auditory brainstem responses. Standard treatment strategies for bilateral hearing loss such as hearing aids are of little use to these individuals. When this disorder strikes young children or infants, it can cause severe disruption of normal language and speech development. The most likely cause of hearing loss is a disorder of the auditory nerve, hence the term "auditory neuropathy." This disorder is rare but more common than previously expected. Investigation of the physiological mechanisms, genetic basis, and possible treatments for this disorder is ongoing.

Endolymphatic Sac Tumors (ELSTs) in von Hippel-Lindau (VHL) Syndrome

NIDCD intramural scientists are studying individuals affected by VHL syndrome and tumors of the inner ear. These ELSTs have been found to develop in approximately 10% of individuals carrying mutations of the *VHL* gene. Hearing loss, balance disturbances, and tinnitus represent the primary clinical manifestations of this disease. Recent molecular genetic studies have confirmed the phenotypic association of ELSTs with VHL syndrome by demonstrating loss of heterozygosity at the *VHL* locus in tumor cells obtained from surgical specimens. In a clinical trial to preserve hearing in individuals with early-stage ELSTs, preliminary results show that these tumors can be safely removed while preserving hearing at preoperative levels and maintaining or improving vestibular function.

Prospective studies of this population of individuals should provide insight into the natural history of hearing and balance disturbances associated with ELSTs, whereas basic investigations will focus on the mechanisms by which ELSTs cause hearing and balance dysfunction.

Large Vestibular Aqueduct Syndrome (LVAS)

LVAS is characterized by progressive childhood sensorineural hearing loss in association with enlarged vestibular aqueducts. Recent data indicate that at least some cases are associated with mutations in the Pendred syndrome gene *(PDS)*. Individuals with Pendred syndrome have sensorineural deafness and goiter. NIDCD intramural scientists are looking for the genetic basis of LVAS, including in several cases in which it is clearly not caused by *PDS* mutations. The role of congenital cytomegalovirus infection in this form of hearing loss is also being studied.

Hereditary Cerebellar Ataxia Syndrome of Early Onset

Several abnormal genes that are associated with inherited cerebellar syndromes that cause balance and coordination disorders have been identified. Relatively little is known about the ways in which different mutations lead to specific types of the disorder. Typically, great differences exist in the clinical signs and symptoms within families that segregate the same mutation and across families with mutations in the same gene. NIDCD-supported scientists have demonstrated linkage to chromosome 19p in four families with episodic vertigo and inability to coordinate muscle movement (ataxia). The scientists identified a missense mutation in the calcium-channel gene on chromosome 19p in a family with severe early-onset progressive cerebellar ataxia involving the trunk, the limbs, and speech function.

Recent research identified several related ataxias associated with different mutations in the same calcium-channel gene. Mutations in other cal

cium-channel genes have been associated with inherited ataxias.

Olfactory Function

NIDCD-supported scientists are investigating relationships between decreased olfactory function and several rare diseases. The studies have shown that olfactory loss appears to be among the first signs of common neurodegenerative diseases such as Alzheimer and Parkinson diseases. Recent psychophysical studies evaluated the prevalence and magnitude of olfactory loss in subtypes of Parkinson disease, Down syndrome, schizophrenia, multiple sclerosis, amyotrophic lateral sclerosis (ALS), and the rare ALS/parkinsonism/dementia complex of Guam. Better understanding of the associations between olfactory function and rare diseases may lead to earlier diagnosis and improved monitoring.

Kallmann Syndrome

Kallmann syndrome is a rare genetic disorder with two main symptoms: an inability to smell and failure of the gonads to mature. This syndrome has a five- to sevenfold greater chance of occurring in males than in females, suggesting that the X-linked form of the disease is the most frequent. NIDCD-supported research has led to the identification of a common developmental defect in nerve migration, which links the two major disease symptoms. NIDCD-supported scientists are investigating a unique family of proteins and their receptors that regulate nerve migration and direction during development. Additional research is focused on isolating and cloning an X-linked gene responsible for Kallmann syndrome.

Papilloma and Carcinoma of Vocal Tract

Papillomas and carcinomas are the most important cancers affecting the human vocal and speech tract. Carcinomas of the upper airway and vocal tract have affected the lives of more than 320,000 Americans and lead to more than 12,000 deaths annually in the United States. Human papillomaviruses are a major cause of these recurrent tumors. NIDCD intramural scientists are collaborating with NIDCD-supported molecular biologists, virologists, and clinicians to address the molecular basis for the disease and possible new treatments. These tumors show an increased response to growth factors compared with other cells. The specific intracellular signaling molecules that mediate this effect have been identified. In addition, these tumors produce factors that stimulate the blood supply and immune cells in ways that promote tumor growth and spread. Drugs that block the effects of these factors may be a new approach for prevention and therapy of these cancers.

Multiple recurrences of these respiratory papillomas and the importance of immune function in controlling viral infection have led to studies demonstrating that affected individuals have a normal immune response to most infections but a suppressed immune response to papillomaviruses. Additional therapeutic approaches that are being tested include photodynamic therapy with the light-sensitive drug Foscan as an alternative to surgery.

Velocardiofacial Syndrome (VCFS) (DiGeorge Syndrome)

VCFS is a disorder that has been associated with more than 30 characteristics, the most common being cleft palate, heart defects, characteristic facial features, minor learning problems, and speech and feeding problems. VCFS is also known as Shprintzen, DiGeorge, cardiofacial, or conotruncal anomaly unusual face syndrome. These syndromes result from a large deletion at chromosome 22q11. VCFS is inherited in only about 10–15% of cases; however, in most instances, neither parent has the syndrome or carries the defective gene, and the cause of the deletion in the affected child is unknown. NIDCD-supported scientists have completed a detailed sequence analysis of the DiGeorge chromosomal region of chromosome 22q11. The 22q11.2 deletion occurs more frequently than originally anticipated, and the endpoints of the deletions occur in clusters. There is considerable variability in the abnormalities associated with deletions of similar size. The presence of a deletion is not always sufficient to cause cleft palate, strongly suggesting that modifier genes interact with the genes of the deletion region. Recent research has

shown that the clathrin heavy-chain-like gene is a strong candidate gene for VCFS.

Williams Syndrome (WMS)

WMS is a rare genetic disorder in children characterized by various distinctive facial features (e.g., cardiac and dental anomalies, hypercalcemia), mental retardation, and a unique behavioral profile of language abilities for individuals with severe general cognitive deficits. This disorder occurs in about 1 in 25,000 live births. NIDCD-supported studies of young children with WMS have documented extreme retardation early on in all developmental milestones, including language. Results suggest that different cognitive domains in WMS (language, spatial cognition, affect) have different starting points and different trajectories unlike patterns discerned in individuals without the disorder and that some aspects of brain organization (e.g., cerebellar abnormalities) are present from a very young age.

Research Initiatives

Cooperative Research and Development Agreement (CRADA) for New Drugs Against Cancers of the Vocal Tract

New drugs that target the specific molecular abnormalities that cause cancers involving the vocal tract are being studied. NIDCD and NCI are collaborating on a 2-year phase I trial of a new drug to be given in combination with radiation for treatment of patients with cancers of the vocal tract (head and neck). The scientists discovered that a signal, nuclear factor κB (NF-κB), is permanently switched on in head and neck cancer cells, activating other genes that cause these cancers to grow. Using cancer cells grown in the laboratory or in mice, scientists were successful in blocking the activation of NF-κB with an investigational drug, PS-341, and inhibiting survival and growth of cancer cells. These studies were supported by a CRADA between NIH and Millennium Pharmaceuticals, the company that produces PS-341. Studies to identify the genes activated by NF-κB and that cause these cancers are also under way so that new tests may be developed for diagnosing and selecting the best therapy for individuals with head and neck cancer.

National Institute of Dental and Craniofacial Research (NIDCR)

Overview

NIDCR's mission is to improve and promote dental, oral, and craniofacial health through research and research training. To do this, NIDCR's programs encompass basic and clinical studies of the broad range of diseases, disorders, and syndromes involving the oral cavity and craniofacial structures; related developmental biology studies; applied research on biologically compatible and biomimetic materials to reengineer damaged or dysfunctional tissues; and behavioral and epidemiological studies to better assess the scope of the problem, identify risk factors and biomarkers for disease, understand health disparities, and provide the knowledge base for improved preventive and health care. Rare diseases and syndromes, such as cancers of the head and neck, orofacial clefting syndromes, early-onset periodontitis, ectodermal dysplasias, craniosynostosis, noma, and disorders of tooth and bone formation such as osteogenesis, amelogenesis, and dentinogenesis imperfecta comprise a significant portion of NIDCR's program activities.

Recent Scientific Advances

Hereditary Gingival Fibromatosis (HGF)

HGF is a rare autosomal disease characterized by a benign but slowly progressive overgrowth and fibrous enlargement of the oral masticatory mucosa. In severe cases, it can prevent tooth eruption and can predispose to chronic infection and tooth recession. The genetic basis for HGF in humans is heterogeneous. HGF may be transmitted as a mendelian trait; the genetic loci for the autosomal dominant forms of HGF have been localized to chromosomes 2p21–p22 (HGF-1) and 5q13–q22 (HGF-2). A team led by an NIDCR-supported researcher has for the first time identified a mutation in the son of sevenless-1 *(SOS1)* gene as the cause of HGF-1. The mutation creates a premature stop codon that results in a chimeric molecule consisting of 1,083 SOS1 NH_2-terminal amino acids followed by 22 replaced amino acids in the COOH-terminal domain. The mutation abolishes four functionally important proline-rich SH3-binding domains normally present in the COOH-terminal domain.

Alterations in the function of SOS1 protein affect a critical biological pathway that influences control of cell cycle, differentiation, and apoptosis. SOS1 has guanine nucleotide-exchange activity and mediates the coupling of receptor tyrosine kinases to Ras activation. The wild-type SOS1 protein is normally maintained in an inactivated state. Truncated mutants lacking either the NH_2-terminal or COOH-terminal domain display guanine nucleotide exchange activity that is significantly higher than the full-length protein, leading to a gain of function. Findings on the mechanisms regulating gingival overgrowth have implications for tissue engineering of gingiva and for understanding the drug-induced form of the disease.

Nonsyndromic Cleft Lip and Palate

Although 70% of oral cleft cases occur as isolated abnormalities; the remaining 30% occur as part of more complex syndromes. An NIDCR-supported scientist is studying the rare autosomal recessive syndrome CLPED1, which is characterized clinically by cleft lip and cleft palate, hypohidrotic ectodermal dysplasia, developmental defects of the teeth and hands, and, in some cases, mental retardation. In contrast to the U.S. population, the prevalence of CLPED1 is high among the population of Margarita Island, Venezuela. By comparing affected and unaffected islanders, researchers previously identified the gene for the CLPED1 syndrome as *PVRL1* on chromosome 11. The investigators have now shown that heterozygote carriers of a mutated *PVRL1* gene have an increased incidence of nonsyndromic clefting. This new finding suggests that other genes known to cause many rare clefting syndromes may also have a

role in the more common nonsyndromic form of clefting.

Amelogenesis Imperfecta

Dental enamel is the hardest tissue in the body and cannot be replaced or repaired because the enamel-secreting cells are lost at tooth eruption. X-linked amelogenesis imperfecta, a phenotypically diverse hereditary disorder affecting enamel development, is caused by deletions or point mutations in the human X-chromosomal amelogenin gene. Although the precise functions of the amelogenin proteins in enamel formation are not well defined, these proteins constitute 90% of enamel's organic matrix. A collaborative study by intramural and extramural NIDCR-funded scientists has shown that disruption of the amelogenin locus to generate amelogenin-null mice results in mice that display abnormal teeth (chalky white discoloration) as early as 2 weeks of age. Microradiography revealed broken tips of incisors and molars, and scanning electron microscopy analysis indicated disorganized hypoplastic enamel. The amelogenin-null phenotype reveals that the amelogenins are apparently not required for initiation of mineral crystal formation but rather for organization of crystal pattern and regulation of enamel thickness. These null mice are a valuable tool for understanding the functions of amelogenin proteins during enamel formation and for developing therapeutic approaches for treating developmental defects affecting enamel.

Hypohidrotic Ectodermal Dysplasia (HED)

NIDCR support has contributed to the discovery of a new gene involved in HED. HED is characterized by defects in ectodermal derivatives such as hair, teeth, and sweat glands. An international team studying a large Middle Eastern family with the disease has identified a mutation in an adaptor protein that relays signals and recruits other proteins to an activated receptor. The genes that have been implicated in HED so far are all part of a conserved developmental

pathway that controls development of hair follicles and teeth in mammals, scales in fish, and, possibly, feathers in birds.

Paget Disease Etiology

Paget disease is the second most common metabolic bone disease that affects 2–3% of the population over age 60. The primary pathological abnormality in patients with Paget disease is increased bone resorption, followed by increased disorganized bone formation that induces bowing of the bone, stress fractures, and arthritis in the involved joints. The cause of Paget disease remains unclear. For more than 30 years, viral infection has been thought to initiate this disease, and several viruses have been implicated (i.e., paramyxovirus, measles virus, respiratory syncytial virus, and canine distemper virus).

NIDCR-supported investigators, using bone marrow cultures to explore the role of viruses in the initiation of Paget disease, have demonstrated that measles virus nucleocapsid transcripts are present in bone marrow cells taken from affected bones of patients with Paget disease. Moreover, mature osteoclasts and osteoclast precursors from Paget disease patients express measles virus nucleocapsid transcripts. Further studies have shown that peripheral blood samples from 90% of patients with Paget disease contain measles virus nucleocapsid transcripts. In vitro infection of osteoclast precursors resulted in an increase in osteoclast size, nuclear number, and bone-resorbing capacity, indicating that measles virus infection enhances osteoclast formation.

Measles virus infection occurs in young patients, whereas Paget disease is a disorder of the elderly. Investigators believe that the initial site of infection occurs in small primitive pluripotent hematopoietic stem cells that remain predominantly in a quiescent phase for a long period. These findings strongly support the hypothesis that viral infection is a potential cause for Paget disease development, and they are a potential basis for designing new strategies for treatment.

Localized Juvenile Periodontitis (LJP)/Early-Onset Periodontitis (EOP)

LJP is a form of EOP that is characterized by rapid loss of alveolar bone localized to the permanent first molar and incisor teeth and has a circumpubertal onset. LJP is a rare form of peri

odontitis; a recent U.S. survey of more than 11,000 adolescents ages 14–17 estimates the prevalence of LJP at 0.53%. Significantly, this survey also indicated that African Americans are nearly 15 times more likely to develop LJP than whites. Cases of LJP tend to cluster within families, suggesting that susceptibility to this form of EOP may be inherited. NIDCR is supporting research on possible genetic defects in the capacity of neutrophil phagocytes to recognize and show directed movement toward microbial peptides. Researchers have discovered polymorphisms in the gene that codes for the neutrophil receptor for these peptides, and other studies have shown that the metabolism and biochemistry of the neutrophils from LJP patients are different from that of healthy controls.

Studies by NIDCR-supported researchers indicate that EOP susceptibility involves the gene for interleukin-1 (IL-1) cytokine on chromosome 2q, a region of chromosome 19q, and additional loci. Researchers are examining the abnormally high IgG2 antibody response in susceptible children that may be inherited. They are specifically testing the hypothesis that the monocyte-derived cytokines, including IL-1, and lipid mediators, such as prostaglandin E_2 and platelet-activating factor, are altered and that the adjusted levels of these soluble factors are responsible for increased production of serum IgG2 and EOP. Antibodies against phosphorylcholine (PC), an antibody that inhibits IgG2 production, appear to be unusually high in EOP patients. EOP may be susceptible to infection because of a decreased chemotactic response of neutrophil phagocytes to normal inflammatory attractants.

LJP appears to be caused by infection with the bacterium *Actinobacillus actinomycetemcomitans (Aa)*. NIDCR-supported researchers are studying immune responses to *Aa* antigens in severe combined immunodeficiency (SCID) mice that harbor human immune cells. Scientists are looking at the clonal diversity of the *Aa* strains and identifying pathogenic clones and the synergy with other microorganisms in dental biofilms (plaque). Several potential pathogenic factors are produced by *Aa,* including a toxin that kills leukocytes, a toxin that kills human epithelial cells and fibroblasts, and factors involved in invasion and adhesion. A new study is using novel techniques to identify in vivo induced antigens, which may serve as targets for vaccine development. NIDCR is supporting genomic sequencing of *Aa,* as well as other studies to develop genetic shuttle vectors between *Aa* and *E. coli.*

New Initiatives

Mechanisms in Nutrition and Infection

Noma is a rare infectious disease associated with abject poverty and severe protein malnutrition. The disease is seen primarily in young children and is characterized by flesh-eating microbial destruction of the orofacial tissues and a mortality rate that exceeds 75%. More research is needed on the mechanisms by which nutritional supplementation can be used to prevent or treat infections, particularly in young individuals. To this end, NIDCR sponsored a workshop on nutrition and oral infections in November 2000 that led to the development of a program announcement, cosponsored by NIAID and NIDDK, to encourage research on biochemical and genetic mechanisms that link nutrition to host resistance to infection.

New Contract Activities

NIDCR issued the broad agency announcement (BAA) Research Registries for the Evaluation of Temporomandibular Joint Implants in February 2002. The purpose of this BAA is to provide researchers and clinicians with high-quality data on patients' medical and clinical histories (e.g., genetic and immunological profile of the enrollees) before and after receiving temporomandibular joint implants and with data on temporomandibular joint implant performance. These objectives will be achieved by supporting one or more awards for registries for a sufficiently large number of temporomandibular joint disorder patients and for a repository of retrieved implants for analysis and storage.

Program Activities

- During FY 2001, NIDCR funded the second year of a 5-year memorandum of agreement with the World Health Organization (WHO) to support the planning and development of protocols and databases for international biomedical, epidemiological, and behavioral collaborative research on craniofacial birth defects, including cleft lip and palate. The goal is to foster collaborative partnerships of international scientists in this research area and to develop a global consensus on the directions and protocols for research on craniofacial anomalies. As part of this major effort, NIDCR and WHO sponsored an international conference in Geneva, Switzerland, in November 2000. More than 80 researchers attended to develop agendas for research on the genetics, gene-environment interactions, and treatment of craniofacial anomalies. At a subsequent meeting held in Park City, Utah, in May 2001, participants discussed research on strategies to prevent craniofacial anomalies.
- A multicenter consortium funded by NIDCR FY 2001 will perform a large-scale genomic screen to identify loci potentially involved in nonsyndromic cleft lip and cleft palate (CL/P). Although the evidence for a genetic component in this disease is substantial, researchers have not yet been able to identify the pattern of inheritance. Evidence suggests that CL/P is a complex trait that may involve as many as 10 genes and involves interactions with environmental factors. The researchers will screen samples from approximately 4,500 individuals in 600 families from four different populations drawn from the United States (Appalachia), South America, China, and the Philippines. The ethnic diversity of the populations is important for modeling the disease against various genetic backgrounds. This project has a strong potential to identify the genes that predispose individuals to CL/P and is expected to set the standard for future studies of complex genetic diseases. The Center for Inherited Disease Research will perform the genotyping.
- NIDCR has funded a new project to identify the genes underlying congenitally missing teeth (hypodontia). This condition typically affects permanent teeth more frequently than primary teeth and results in a bilaterally symmetrical pattern of missing teeth. The treatment of patients affected by hypodontia is complex and expensive and may involve a combination of pediatric dentistry, orthodontics, prosthodontics, and implantology. Congenital hypodontia demonstrates considerable heterogeneity, and, although it is usually transmitted as an autosomal dominant trait with incomplete penetrance and variable expressivity, it may also be transmitted as an autosomal recessive or X-linked trait. This research project will recruit patients and develop phenotypic criteria for distinguishing specific patterns of hypodontia. Genes underlying hypodontia will be identified by genomewide linkage analysis, candidate gene identification and mutation analysis. Genomewide screening will be performed by the Center for Inherited Disease Research.
- NIDCR is supporting 1 of 10 projects awarded in FY 2001 through the Global Network for Women's and Children's Health Research, a joint initiative by NIH and the Gates Foundation. The NIDCR co-supported study will evaluate the impact of nutritional supplementation and smoking cessation on the incidence of oral clefts and neural tube defects in a South American population. A request for applications issued in March 2000 invited applications for the Global Network for Women's and Children's Health Research.

Program Workshops, Symposia, and Meetings

International Conference on Treatment of Salivary Gland Disorders: Alternative Approaches

This 2-day international conference was held in Bethesda, Maryland, in July 2001. The conference was cosponsored by ORD, NCCAM, ORWH, Laclede, Inc., the Food and Drug Ad

ministration, the Office for Women's Health of the Department of Health and Human Services, and Amarillo Biosciences, Inc. The purposes of the conference were to 1) bring together scientists with an interest in basic and clinical research on salivary gland dysfunction and others working in alternative/complementary medicine approaches to exchange information and ideas on new research strategies and advances; 2) enhance interest in elucidating the mechanisms by which alternative medicine approaches, such as acupuncture and traditional Chinese medicine, may work in relieving xerostomia (dryness of the mouth); and 3) create opportunities for research collaborations and interactions among investigators in this field. Sixty-one scientists from the United States, Sweden, Finland, China, Japan, Netherlands, and Mexico attended the conference. Sessions included discussions of research advances in salivary dysfunction, the efficacy of conventional treatments, progress in alternative approaches to therapy, and future directions for research and clinical practice.

Saliva production and secretion are critical to maintain oral health. Many serious complications are induced by salivary gland dysfunction, including increased rate of dental caries, periodontal diseases, bacterial sialadenitis, mucosal infections, and difficulty in swallowing, chewing, and speaking. Salivary gland dysfunctioncan be induced by conditions such as radiation therapy for head and neck cancers, rheumatoid arthritis, autoimmune disease, and viral infections, such as hepatitis and AIDS. Effective therapeutic measures for salivary gland dysfunction are still lacking; current medications include saliva substitutes and secretion stimulants that are mainly aimed at relieving symptoms.

Recent investigations have shown that alternative medical approaches can be effective in treatment of salivary gland dysfunction. Swedish researchers have used acupuncture to alleviate xerostomic symptoms and increase saliva secretion in Sjögren syndrome, radiation therapy, and medication-induced salivary gland dysfunction. Studies delineating the mechanisms found that acupuncture increased local blood flow and the release of several neuropeptides that stimulate saliva secretion, including vasoactive intestinal polypeptide, neuropeptide Y, and calcitonin-related peptide. Another class of alternative medicine, Chinese medicine, is also effective in the treatment of xerostomia induced by Sjögren syndrome, radiation therapy, and aging. The major barrier hindering the use of Chinese medicine in Western countries is a lack of awareness and understanding of its effectiveness and working mechanisms. Further basic studies to investigate the effective component and mechanisms of Chinese medicine and double-blind, randomized clinical trials to evaluate its effectiveness are of great importance. Obviously, more information exchange and collaborative studies will be beneficial. This conference enhanced interest in elucidating the mechanisms by which alternative medicine approaches work in relieving Sjögren syndrome-induced xerostomia, and it created opportunities for research collaborations and interactions among investigators in the field.

National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

Overview

NIDDK supports research on many rare diseases. Although diseases such as type 1 and type 2 diabetes are not rare, some rare single gene defects cause some types of diabetes, such as maturity-onset diabetes of the young, and lipodystrophy. Many of the genes that cause these disorders have recently been identified. In addition, NIDDK supports research on kidney and liver diseases. Every year, 20,000 babies are born with kidney problems, of which 2,000 will die and 1,000 will begin treatment for kidney failure. The Institute also supports research on genetic metabolic diseases, such as cystic fibrosis; lysosomal storage diseases, including Fabry disease, Niemann-Pick disease, and the mucopolysaccharidoses; disorders of copper transport, including Menkes disease and Wilson disease; and hematological diseases, such as Cooley's anemia and sickle cell disease.

Recent Scientific Advances

Fabry disease is a rare genetic disorder caused by a deficiency in the enzyme α-galactosidase A. Because the gene is located on the X chromosome, mostly males are affected (an estimated 2,000–4,000 in the United States), although female carriers of the defective gene may exhibit symptoms of the disease. The enzyme normally helps to break down a fat (lipid) called globotriaosylceramide (GL-3). Without adequate α-galactosidase A, GL-3 accumulates in the cells, tissues, and organs of affected patients, which, over time causes damage to the heart, kidneys, and brain, leading to life-threatening problems. In 2001, investigators demonstrated the feasibility of using enzyme replacement to correct the metabolic defect in Fabry disease. Proof-of-concept studies in a mouse model of the disease were published, and a phase I dose escalation study was performed to demonstrate safety. Two double-blind clinical trials were published; one was conducted at the NIH Clinical Center and the other by the International Collaborative Fabry Disease Study Group. In the latter study, patients were randomly assigned to receive recombinant α-galactosidase A or a placebo. Patients received 11 infusions of recombinant α-galactosidase A over a 20-week period. Heart, kidney, and skin biopsies were examined for deposits of GL-3 both before and during treatment. Investigators were primarily concerned with levels of GL-3 in the kidney, because kidney failure is a major feature of Fabry disease. Approximately 69% of the 29 patients had a return to near-normal levels of GL-3 in their kidneys, and the rest showed significantly decreased levels. The skin and heart were also markedly improved. After the initial 20 weeks, the study was extended into an open-label design that confirmed the results of the double-blind study and showed that the heart, kidneys, and skin remained clear of deposits or that deposits were further reduced over the treatment period. In addition, patients reported improvement in their pain and in their quality of life. All patients elected to continue the treatment after the trial was completed.

Niemann-Pick Disease Type C

Niemann-Pick disease type C (NPC) is an autosomal recessive lipid-storage disorder characterized by progressive deterioration of the central nervous system resulting in early childhood death and affecting an estimated 500 children in the United States. The disease is inherited when a child receives two mutant genes, one from each parent. In NPC, cholesterol derived from low-density lipoprotein accumulates in cells of the brain, liver, spleen, lungs, and bone marrow, leading to an enlarged spleen and liver, poor muscle control, impaired eye movements, slurred speech, and dementia. Previously, researchers identified one of the genes, *NPC-1,* which is responsible for 95% of NPC cases. The NPC-1 protein, located in lysosomal compartments within the cell, was originally expected to play a key role in cholesterol transport; instead, investigators recently determined that the NPC-1 protein resembles a family of bacterial perme

ases that transport various substances throughout the bacterial cell membrane. Researchers now believe that NPC-1 is a permease that can transport lipids such as fatty acids, but apparently not cholesterol, across membranes.

In related work, researchers identified a second gene, *HE1,* which is responsible for the remainder of NPC cases. Disease caused by mutations in *HE1* is sometimes called NPC-2. *HE1* encodes for a cholesterol-binding protein found in many cell types throughout the body. *HE1* was found in normal skin cells but not in skin cells from NPC-2 patients. In addition, researchers found that adding normal HE1 protein to cells derived from NPC-2 patients restores the ability of the abnormal cells to transport cholesterol. Gene sequencing further confirmed that the *HE1* gene from NPC-2 patients contained mutations that made it unable to function. Researchers suspect that NPC-1 and NPC-2 are both required for the appropriate transport of cholesterol.

Mucopolysaccharidosis Type I

Hurler syndrome is the most severe form of mucopolysaccharidosis. It is a recessive genetic disease caused by mutations in the gene for α-L-iduronidase, which degrades the glycosaminoglycans. Up to an estimated two-thirds of patients with Hurler syndrome have premature stop mutations. The aminoglycoside gentamicin has been shown to promote readthrough of stop mutations. This year, researchers have investigated whether gentamicin can increase readthrough in fibroblasts from Hurler patients. The studied fibroblast line is a compound heterozygote with two different stop mutations in the iduronidase gene. In the presence of gentamicin, the cell line showed 2.8% of normal enzyme activity. These cells also showed a reduction in the storage of glycosaminoglycans. This encouraging finding in an in vitro model needs to be reproduced in vivo. The approach could be exploited as a potential treatment for Hurler patients with stop mutations.

Cystic Fibrosis (CF)

CF is the most common fatal genetic disease in whites, affecting approximately 1 in 2,500 newborns. Patients are diagnosed in early childhood based on symptoms such as failure to thrive. By managing the nutrition problems and infections associated with CF, the life expectancy for a CF patient has increased to 30 years. Since the cloning of the CF gene and identification of its protein product, CFTR, as a cyclic AMP-regulated chloride channel, impressive progress has been made in the molecular understanding of this disorder. NIDDK supports a research portfolio directed at further defining the molecular mechanisms underlying CF and translating information about the molecular basis of the disease into new treatments. This year, investigators have demonstrated a new function for CFTR to transport bicarbonate. Mutations that altered bicarbonate transport were clustered in the first half of CFTR. Molecules with aberrant bicarbonate transport occurred in patients with pancreatic insufficiency. The alteration of bicarbonate can lead to fluid that is acidic and may explain some of the diverse pathology of the disease. In addition, this finding demonstrates that correction of the chloride transport defect is not likely to correct the symptoms of this disease.

Pseudohypoparathyroidism (PHP) Type 1b

PHP is a dominantly inherited genetic disease characterized by short stature, mental retardation, and bone abnormalities. PHP type 1 has two genetically distinct forms. In PHP type 1a, heterozygous defects in the *GNAS1* gene lead to reduced expression or activity of the α-subunit of the G protein Gsa, which couples many G protein receptors to activation of adenylate cyclase. PHP 1a is associated with resistance parathyroid hormone (PTH) and other hormones that act through activation of adenylate cyclase. In PHP type 1b, a more rare form of the disease, hormone resistance is limited to PTH target tissues such as kidneys. PHP 1b has been linked to chromosome 20q13, a region containing the *GNAS1* gene. *GNAS1* has three alternatively spliced first exons that are imprinted. In PHP 1b, maternal *GNAS1* alleles are defectively imprinted. This defect may suppress expression of *GNAS1* from the maternal allele in affected tissues, such as kidneys, and lead to complete absence of Gsa protein and PTH resistance. Cur

rent work focuses on understanding the basis for the imprinting defect.

Peroxisomal Biogenesis Disorders (PBDs)

PBDs are a genetically heterogeneous group of rare human diseases, including Zellweger syndrome, neonatal adrenoleukodystrophy, infantile Refsum disease, and rhizomelic chondrodysplasia punctata. These diseases often result in neurological, hepatic, and renal abnormalities; pronounced mental retardation; and death in childhood. PBDs result from an inability to properly import the many enzymes required for processes such as β-oxidation of fatty acids and synthesis of bile acids, cholesterol, and plasmalogens into peroxisomes. The enzymes responsible for these important metabolic functions are synthesized in the cytoplasm and then are posttranslationally imported into the peroxisome, where they function. More than 16 proteins have been identified and shown to be required for peroxisome biogenesis and import. Using novel experimental approaches, investigators have recently demonstrated that the receptors importing certain enzymes into the peroxisome are subsequently recycled from the inside of the organelle back to the cytosol, where they then undergo additional rounds of transport. This unusual shuttling mechanism distinguishes protein import into peroxisomes from that of most other organelles, with the exception of the nucleus. NIDDK investigators continue to actively elucidate the mechanism and the cellular proteins involved in the recognition, targeting, and translocation of enzymes into peroxisomes, with the ultimate goal of creating therapeutics to alter the progression of the disease processes in PBDs.

Menkes Disease and Wilson Disease

Menkes disease and Wilson disease result from aberrant copper transport. In Menkes disease, patients are unable to transport copper, resulting in a deficiency of copper, which is an important cofactor for many enzymes. Children with Menkes disease suffer from growth retardation, neurological impairment, seizures, and hair and bone abnormalities leading to death by age 5. Wilson disease is an adult-onset disease resulting from an excess of copper. Patients suffer from liver failure and brain deterioration due to copper deposits in those organs. New insights into copper transport inside the cells have been gained through recent identification of metallochaperones, a family of proteins that play a role in this transport process. The delivery of copper to intracellular targets in mammals appears to require the metallochaperone *Atox1*. Investigators have studied the role of this new protein by creating a mouse model where the *Atox1* gene has been knocked out. Most of these mice did not survive long after birth, and those that did survive exhibited growth failure, flaccid skin, and seizures due to copper deficiency. These findings are consistent with the role of *Atox1* interacting with both Menkes and Wilson copper transport proteins. The studies suggest that *Atox1* plays an important role in delivering intracellular copper for export from the cell.

Research Initiatives

NIDDK published a request for applications (RFA) on June 28, 2001, for the Biliary Atresia Clinical Research Consortium, which will be funded in summer 2002. The consortium will establish a network of 5–10 clinical centers and a data-coordinating center to aid in expanding research in biliary atresia and neonatal hepatitis. A database of clinical information and a serum and tissue repository have been proposed to assist in developing hypotheses and generating research topics and protocols about pathogenesis of biliary atresia and neonatal hepatitis. In addition, the consortium will direct the development of clinical trials to establish optimal management and therapy of children with biliary atresia and neonatal hepatitis.

NIDDK issued the program announcement (PA) Research Studies on Hereditary Oxalate Stone Disease. The purpose of this PA is to increase investigator interest in research into the genetics and heritability of oxalate regulation and the oxalate stone diseases. In several known inherited disorders, affected individuals develop recurrent calcium oxalate stones at a very early age. These individuals have a genetic metabolic disorder known as primary hyperoxaluria, in which a genetic defect in the regulation of oxalate synthesis in the liver causes excessive ex

cretion of oxalate in the urine and excessive accumulation of oxalate. This PA sets aside funds for pilot and feasibility studies that use new and innovative approaches to the study of these disorders. A single grant application was received in FY 2001. Eight additional applications have already been received in FY 2002, and two have been funded to date.

NIDDK issued an RFA to recompete the CF core centers. The purpose of this program is to provide core resources to investigators studying CF. Centers also provide an enrichment program to bring in outside expertise on CF. NIDDK funded one center in response to this initiative.

NHLBI and NIDDK cosponsored the RFA Transactivation of Fetal Hemoglobin Genes for Treatment of Sickle Cell Disease and Cooley's Anemia, which supports the pursuit of the unambiguous identification of transactivator proteins that regulate the expression of fetal globin chains. To further understanding of the *trans*-acting component of developmental stage-specific hemoglobin isoform switching, validation of existing candidate transactivators and gene discovery to identify new ones were encouraged. Finally, these targets will form the foundation of new drug-based and/or genetic approaches to reinduction of fetal hemoglobin in adult clients with sickle cell disease or Cooley's anemia to ultimately provide universal cures for β-chain hemoglobinopathies such as sickle cell disease and Cooley's anemia. NIDDK funded four projects under this RFA.

NIDDK cosponsored the RFA Genetic Modifiers of Single Gene Defect Diseases with NHLBI. The purpose of this RFA was to support research to identify genetic modifiers that affect the phenotype of genetic diseases. All diseases are variable in their presentation because of differences in the genetic makeup and environmental exposure of the affected individual. For disorders inherited in a mendelian fashion, a single gene plays the predominant role in the development of a disease phenotype. However, phenotype variation occurs even among those who have identical genotypes at a disease locus. Other genes that contribute to phenotype variability must be found to further understanding of the molecular basis of monogenic disorders. NIDDK funded two grants from this RFA that proposed to identify modifier genes in CF and thalassemia.

Program Activities

NIDDK and ORD sponsored the First International Workshop on Lipoatrophic Diabetes and Other Syndromes of Lipodystrophy at NIH on March 22–23, 2001. The workshop discussed the genetic etiologies of these syndromes and the available mouse models in which to study these disorders. One highlight during this workshop was the report that treatment of severe generalized lipoatrophy with leptin results in clinical benefit.

An international workshop, Noninvasive Measurement of Iron, organized by NIDDK and cosponsored by ORD, was held on April 17, 2001. Its purposes were to assess the current state of science and to identify areas for further investigation. The workshop revealed a clear clinical need for quantitative means of measuring body-storage iron that are noninvasive, safe, accurate, and readily available to improve the diagnosis and management of patients with iron overload, including hereditary hemochromatosis, thalassemia major, sickle cell disease, aplastic anemia, and myelodysplasia. Magnetic resonance imaging (MRI) is the best available technique for examining the three-dimensional distribution of excess iron in the body, but further research is needed to develop means to make quantitative measurements. Currently, biomagnetic susceptometry is the only noninvasive method for measurement of tissue iron stores that has been calibrated, validated, and used in clinical studies; however, the complexity, cost, and technical demands of the liquid-helium-cooled superconducting instruments have restricted clinical access to the method. The workshop identified basic and clinical research opportunities to increase understanding of the physical properties of iron and iron toxicity, further investigate MRI as a method for quantitative determinations of tissue iron (especially in liver and brain), and develop improved methods and more widely available instrumentation for biomagnetic susceptometry.

NIDDK and ORD provided partial support for a meeting organized by the National MPS Society (a family support group)—Strategies for Therapy of MPS and Related Diseases—held June 21–24, 2001, in Los Angeles, California. This meeting was held concurrently with the 16th annual conference of the National MPS Society, enabling investigators and families to attend each others' sessions. The purpose of the meeting was to discuss progress and challenges in understanding and treating this group of disorders. Several new animal models for MPS were described to provide at least one model for each form of MPS. Progress was reported in the treatment of these disorders, and several clinical trials for enzyme replacement therapy are ongoing or planned.

National Institute on Drug Abuse (NIDA)

Overview

NIDA provides national leadership and conducts and supports biomedical and behavioral research, health services research, research training, and health information dissemination with respect to the prevention of drug abuse and treatment of drug abusers. NIDA plans, conducts, fosters, and supports a comprehensive program of research and research training relating to the causes, prevention, treatment, patterns, and consequences of drug abuse and addiction through research performed in its own laboratories and through contracts and grants to both scientific institutions and individuals. NIDA supports training in fundamental sciences and clinical disciplines relating to drug abuse by individual and institutional research training awards and coordinates with other research institutes and Federal health and other agencies on activities relevant to drug abuse and addiction. NIDA conducts and fosters health information dissemination activities, including the collection and dissemination of research findings and related educational materials for health professionals, educators, and the public. In addition, NIDA coordinates with institutions, professional associations, and international, national, state, and voluntary agencies working in these areas, including collaboration with the Substance Abuse and Mental Health Services Administration (SAMHSA), on services research and other program issues.

History

Currently, four drug abuse treatment medications have received orphan product designation: levomethadyl acetate hydrochloride, naltrexone, buprenorphine, and naloxone. Levomethadyl acetate hydrochloride (Orlaam), an alternative to methadone used for opiate maintenance therapy, received new drug application (NDA) approval in 1993. Naltrexone, an opiate antagonist for use in detoxified patients, was approved in 1985 and no longer enjoys orphan exclusivity. The opiate partial agonist buprenorphine and a combination of buprenorphine plus naloxone have also received orphan designation (see details below) but do not currently have approved NDAs.

Incidence and prevalence figures for dependence on controlled substances (not including alcohol or nicotine) are always difficult to estimate because they vary from type of drug, community, and supply availability (generally a function of supply interdiction or law enforcement). Unlike other disease conditions, illicit marketers have a reason (profit) to introduce and infect the population with abusable and/or dependence-producing substances. Illicit drugs used in some communities are not always available or in vogue in other communities; thus, various drug dependence indications may themselves affect fewer than 200,000 people in the United States. Clearly, however, abuse of opiates (heroin and other narcotics) and stimulants (e.g., cocaine and crack cocaine) is endemic in the United States. Even the lowest estimates put dependence levels of these substances at figures well above the 200,000 threshold generally used for defining orphan products. The total disease burden of drug abuse in the United States has been estimated to exceed $160 billion per year.

Additionally, injection drug use and sexual contact among users is a highly correlated vector in the spread of human immunodeficiency virus (HIV), hepatitis, and tuberculosis. These illnesses create a public health problem of enormous magnitude while being treated as orphan diseases by the pharmaceutical industry.

Despite the public health burden of this disease state, pharmaceutical companies have little or no incentive to pursue research and development of new treatment medications for this population. Although the total number of people afflicted may seem sufficient in the aggregate, unlike other disease states, many of these people are not seeking treatment at the same time. Therefore, the actual population who may be a potential market for medications is actually only a fraction of the total number who could benefit. Additionally, many people will be treated in

publicly funded clinics where companies perceive reimbursement as modest or inadequate and subject to artificial control. Some treatment agents may themselves be abusable and will be strictly controlled (e.g., methadone, classified as a schedule II controlled substance for use in opiate maintenance therapy—some 900 U.S. clinics are licensed to dispense methadone, serving approximately 190,000 people per year, with a pharmaceutical market value of approximately $30 million per year). This is simply not an attractive market to most manufacturers based on projected return on investment compared to nearly any other indication they could pursue. Each of these points is well documented in the recent Institute of Medicine Report on the Development of Medications for the Treatment of Opiate and Cocaine Addiction, 1995, and each is well known to the pharmaceutical and market research industries.

Finally, pharmacological treatment of drug-dependent populations is not the dominant treatment modality in the United States. Most therapeutic regimens are nonpharmacologically based. Because no medications have demonstrated efficacy for the treatment of cocaine dependence, NIDA's orphan product experience to date has focused on medications to treat opiate dependence.

Therefore, while de jure opiate and cocaine dependence do not fit the definition of orphan products, de facto they do. For example, consider the development and approval of Orlaam, an alternative to methadone. Despite the facts that human data on 6,000 subjects from government-sponsored studies were available for levomethadyl acetate hydrochloride, that the compound was off-patent, and that the government had a large supply of the compound available for anyone interested in obtaining an NDA, no private-sector entity attempted to finish the development of this compound until NIDA paid a contractor to do so. Similarly, the development of naltrexone was largely a NIDA-funded effort. Therefore, these products should be viewed as orphanlike in their ability to attract private-sector sponsors, and, until recently, this was also persuasive to the Food and Drug Administration (FDA).

In the case of pharmacological treatment for opiate dependence, the population in treatment currently cannot exceed 190,000 per year. Historically orphan designation was permitted for two products substantially developed by NIDA (naltrexone, 1985, and Orlaam, 1993) as treatments for opiate dependence. Orlaam received orphan designation because it could be used to transfer patients from methadone (and fewer than 200,000 people were receiving methadone), and naltrexone received designation based because there were fewer than 200,000 detoxified addicts at any given time.

In the more recent designation of buprenorphine (1994), the FDA appeared to be taking a more restrictive view via application of their more recently promulgated regulations. Although the issue was not definitively answered in the case of buprenorphine, the FDA expressed the view that orphan designation in which the population in question exceeded 200,000 people would be difficult unless there was a medical/biological reason why 200,000 people could not use the product. In other words, treatment capacity (the number of individuals seeking treatment) might not suffice for orphan designation. In the case of buprenorphine, this did not prove to be an insurmountable burden, because that product's sponsor could prove to the FDA that it would not recoup its investment during 7 years of exclusive marketing in the United States according to historic, current, and projected expenditures. Thus, a unique situation exists where the preclinical efforts are completed, NIDA is supporting clinical trials, and the sponsor has the expertise to manufacture new formulations. Buprenorphine became the first product to receive an orphan designation based on an economic- rather than a population-based rationale. The sponsor selected this route because there was less certainty that the FDA would continue to allow orphan designation based on the capacity of the treatment system as opposed to the actual incidence and prevalence of opiate dependence.

Recent Scientific Advances

The discovery of opiate receptors by NIDA-funded scientists in the 1970s opened a new era of neurobiological research that is ongoing. Sci

entists continue to map brain receptor system types and subtypes, continuously gaining understanding of their structure and function. This information will enable the design of interventions (behavioral, chemical, and genetic) that may be useful to treat myriad disorders, all of which are mediated in the brain.

A generation of research has shown that drug addiction is a complex biomedical and behavioral disease that has its roots in the parts of the brain that underlie, mediate, and allow us to have the emotions that make us human. Just as depression is a brain disease that can be treated with medicine, drug addiction is a brain disease that can and should be treated with medicine.

A critical distinction exists between drug abuse and drug addiction. Drug abuse is a voluntary behavior; the casual user makes a free and conscious decision to break the law and use an illicit, mind-altering substance. Drug addiction is a disease of the brain, resulting from repeated and prolonged self-administration of such a substance. Addiction is brought on by drug-taking behavior in much the same sense that lung cancer is brought on by cigarette smoking and heart disease is brought on by excessive fat intake. Once the disease is established, however, whether in the brain, lungs, or heart, the physiological dysfunction must be corrected to restore health.

The role of medication is to reestablish normality to brain function and behavior so that the addicted patient has the opportunity for rehabilitation through counseling, psychotherapy, vocational training, and other therapeutic services. Although the mechanisms of many central nervous system (CNS) disorders are still to be elucidated, scientists working in the field of drug abuse have now identified and cloned the putative site of action in the brain for every major drug of abuse. Thus, the potential to develop new treatments is enormous. For example, having cloned the dopamine (DA) transporter mechanism where cocaine exerts its action, NIDA scientists are now designing molecules that will block cocaine's effects at this site without disrupting essential neurotransmitter functions of DA.

Additionally, NIDA and other scientists have developed pharmacological agents for the treatment of opiate dependence in various functional categories. For example, methadone and Orlaam are μ-agonist medications currently approved for opiate treatment. Naltrexone is an opiate antagonist approved for treatment, and naloxone is approved for treatment of opiate overdose. NIDA is working on a partial μ-agonist (buprenorphine) that will further contribute to the arsenal of agents available for treatment.

Research Initiatives

As described in the history section, NIDA considers medications for the treatment of dependence on controlled substances to be de facto orphans. Thus, the development of medications for the treatment of these conditions may be considered as rare disease research within the context of an urgent public health need with a wholly inadequate private-sector response. Therefore, NIDA's medications development program (MDP) effort may be considered part of a rare disease research initiative (until facts prove otherwise).

In 1990, the McCune-Albright Syndrome (MDD) was established in NIDA. In 1999, MDD became part of the Division of Treatment Research and Development (DTR&D). The functions of MDD within the new division remained the same; namely, DTR&D conducts studies necessary to identify, develop, and obtain FDA marketing approval for new medications for treatment of drug addiction and other brain and behavior disorders; develops and administers a national program of basic and clinical pharmacological research designed to develop innovative biological and pharmacological treatment approaches; supports training in fundamental sciences and clinical disciplines related to the pharmacotherapeutic treatment of drug abuse; collaborates with the pharmaceutical and chemical industries in the United States and other nations and the Federal MDPs of other institutes and entities; and works closely with FDA to ensure that research designed to show the clinical efficacy of new compounds is evaluated and approved in the most expeditious manner possible.

The division operates within the larger context of a NIDA-wide medications development program that incorporates basic research discoveries from other divisions (intramural and extramural) in the quest to develop new pharmacological treatments. Application of research results from the intramural and extramural community gives the division access to the latest theoretical bases and an opportunity to test new hypotheses in controlled clinical settings.

Recognizing that physicians will soon have a choice of five different FDA-approved products for treating opiate addiction (methadone, Orlaam, buprenorphine, buprenorphine-naloxone, and naltrexone) and no FDA-approved products for treating addictions to stimulants (e.g., cocaine and methamphetamine), NIDA's efforts are shifting toward a greater emphasis on discovery and development of medications for treating stimulant dependence. Although initial clinical trials in this area have focused on medications already marketed for other indications, substantial efforts are being made to discover and develop novel compounds that may specifically address the problem of stimulant dependence. Note that within the pharmaceutical industry, approximately $75 million is devoted to biological screening and pharmacological testing for each successful new medication that reaches the market. NIDA is willing to make such an investment in medications discovery and is planning to collaborate with pharmaceutical companies that will allow NIDA to screen chemical libraries at biochemical targets (e.g., DA D_1 receptors) implicated as potential sites of action for effective medications. Efforts are also directed toward supporting, through grants and contracts, synthesis of novel compounds for screening and pharmacological testing.

Significant areas of research and development are summarized below.

Opiate Addiction Treatment

Buprenorphine/Buprenorphine-Naloxone Combination

A 16-week, 735-patient, 12-center trial of buprenorphine, an opiate partial agonist medication for the treatment of heroin dependence, has been completed. Data from this study, as well as other previous and ongoing studies, have been analyzed. These data demonstrate the effectiveness of buprenorphine in reducing illicit opiate abuse and in retaining patients in treatment.

Under development is a combination dosage of buprenorphine plus naloxone that would be useful as a potential nonnarcotic or "take home" treatment medication. Testing is under way to determine the abuse potential of buprenorphine plus naloxone. A tablet form of buprenorphine and of buprenorphine plus naloxone is also under development, and commercial sponsorship for these products has been negotiated. A new drug application (NDA) for buprenorphine was submitted in 1997, and a 12-site, 52-week trial of the tablet products was completed in September 1998. The NDA for buprenorphine combined with naloxone was submitted and reviewed in 1999. The FDA has deemed both submissions approvable, and actual approval may occur in 2002.

Given that buprenorphine plus naloxone received an approvable status by the FDA in calendar year 1999, plans are under way to enhance the introduction of this new frontline product to the American treatment system. The hope is that pharmacological treatment with buprenorphine products will enable it to be used in more settings than is currently the case for methadone and Orlaam. To test the viability of this approach, NIDA and the Department of Veterans Affairs (VA) began a study in 1999 designed to assess the use of a buprenorphine-naloxone sublingual tablet formulation in nonclinic-based settings for the treatment of opiate addiction. The study also evaluated the administration of buprenorphine to adolescents (ages 15–21 years). A total of 583 patients were enrolled, and the study was completed in February 2002.

Data from this study are being analyzed; they will be used to expand labeling and will be considered in developing guidelines for the administration of the buprenorphine-naloxone sublingual tablet in office settings. The study was implemented in university-based or -affiliated clinics, community mental health clinics, or private physicians' offices—with buprenorphine

prescribed and supplemented with relapse prevention therapy delivered by an appropriately trained medical assistant. A collaborative arrangement of this protocol allowed physicians practicing in one of the above settings to participate in this study provided they agreed to adhere to the requirements of the protocol. The study was specifically aimed at answering the following critical treatment expansion issues:

- Determine and document the safety of extending buprenorphine treatment to a younger population (ages 15–21 years).
- Document physicians' patterns of preferred prescribing practices, including induction, dose adjustment, maintenance, and take-home dosing.
- Document the ease or difficulty physicians encounter in delivering buprenorphine treatment in various treatment settings, the necessary accommodations, and the advantages/disadvantages of this treatment strategy (from the physicians' perspective).
- Document the acceptance, compliance, preferences, and necessary adjustments from the patients' perspective.
- Document treatment education issues regarding consideration of physician guidance materials or future coursework by professional societies after anticipated FDA approval.

Depot Naltrexone

Naltrexone, a marketed long-acting, orally effective opioid antagonist, was approved in 1985 to block the pharmacological effects of exogenously administered opiates. It is an adjunct to the maintenance of the opioid-free state in detoxified, formerly opioid-dependent individuals.

One of the major obstacles to the success of naltrexone has been patient compliance with therapy. Naltrexone must be taken at least three times per week and has no effect other than to block the effects of heroin, a drug that the patient is not supposed to use. Because of this, many patients forget to take or stop taking their medication. Therefore, the greatest success with naltrexone has been in the limited population of highly motivated formerly opiate-dependent patients.

During 1999, NIDA completed the production and preclinical testing of a batch of 120 doses of depot naltrexone, via a Small Business Innovative Research (SBIR) grant to Biotek, Inc. These doses are designed to last 30 days when administered subcutaneously in humans and produce a blood level of about 2–3 ng/ml (which is relatively constant over this period). This product has been shown in an inpatient clinical study to block subjective responses to heroin challenges at 12–25 mg. The study was completed in 2000 and showed that 25-mg heroin challenges can be blocked up to 5 weeks after depot injection. A two-site outpatient double-blind study was designed to test the product in a real-world setting. This outpatient study began in November 2000 and is expected to be completed by the end of 2002.

Another investigational formulation of a sustained release formulation of naltrexone supplied by Alkermes, Inc., is undergoing clinical trials at NIDA's intramural research program. This study will provide additional information on the safety and duration of efficacy of this potential treatment product.

Thus, the feasibility of a sustained-release formulation of naltrexone for the treatment of opiate addiction is moving rapidly from concept to clinical testing. Naltrexone was approved in 1994 for the treatment of alcohol abuse; the depot preparation may also be of value for the treatment of that disease.

Opioid Peptides as Medications

There are three major types of opioid receptors in the brain: μ, δ, and κ. Morphine, heroin, methadone, and Orlaam bind to the μ-receptor with high affinity. The naturally occurring ligand for the κ-opioid receptor is dynorphin A. Animal studies indicate that dynorphin A alleviates opiate withdrawal and decreases tolerance to chronically administered μ-opioid receptor agonists (e.g., morphine). Indeed, κ-opioid abnormalities in the CNS may underlie the drug

seeking behavior of heroin addicts. NIDA-funded scientists are investigating the effects of dynorphin on opiate withdrawal in opiate-dependent subjects.

Cocaine Addiction Treatment

Compounds in Advanced Clinical Testing

Several small studies of potential cocaine addiction treatment agents have been completed and are in various stages of data analysis. Clinically significant findings will be followed up in larger controlled trials as warranted. The development of anticocaine medications has proved a daunting task; to date, only one potential medication justifies a phase III trial. A retrospective analysis of a phase II trial of selegiline in cocaine addicts indicated a differential outcome in favor of selegiline versus placebo. However, this finding needs to be replicated in a larger and more statistically powerful clinical trial. DTR&D is conducting a phase III multicenter trial of selegiline that began in spring 2001.

Additionally, a growing body of evidence generated by NIDA grantees concerns the potential use of disulfiram in the treatment of cocaine dependence. Disulfiram (Antabuse), marketed as aversive therapy for treating alcoholism, shows promise in the treatment of cocaine dependence. Several NIDA-sponsored studies conducted at Yale University documented interaction of disulfiram with cocaine in humans. Pharmacokinetics studies showed that disulfiram increases plasma concentrations of cocaine and potentiates physiological cardiovascular responses to cocaine. Three efficacy trials conducted with different populations of cocaine-dependent individuals suggest that disulfiram combined with each of three different therapeutic interventions (cognitive behavioral treatment, 12-step facilitation, or clinical management) might be effective in treating cocaine dependence. In cocaine-alcohol abusers, disulfiram treatment showed sustained effect on reduced cocaine and alcohol use 1 year after cessation of therapy. Disulfiram treatment of cocaine-abusing opioid-dependent patients maintained on methadone resulted in a significant decrease in the amount and frequency of cocaine use. A preliminary study showed that disulfiram also decreases cocaine use in cocaine-abusing opioid-dependent addicts maintained on buprenorphine.

The efficacy of disulfiram in treatment of cocaine dependence might be related to its effect on increases in cocaine-induced nervousness, anxiety, and high and may result from alteration of cocaine craving mediated by the relative excess in brain DA and decrease in noradrenaline resulting from inhibition of the enzyme β-hydroxylase. In the population of alcohol-abusing cocaine addicts, disulfiram may block the antianxiety effects of alcohol, which generally leads to increased use of cocaine.

NIDA is sponsoring three large outpatient clinical trials with disulfiram as the treatment for cocaine dependence: 1) 160 opioid-cocaine-dependent patients maintained on methadone, conducted at Yale University; 2) 180 opioid-cocaine-dependent patients maintained on buprenorphine, conducted at Yale University; and 3) 208 alcohol-cocaine-dependent individuals maintained on disulfiram and naltrexone alone and in combination, conducted at the University of Pennsylvania. All of these studies include some form of behavioral or cognitive therapy and drug counseling. They are monitoring not only cocaine use but also use of opiates or alcohol. Finally, NIDA is planning a clinical pharmacology/safety study of the interactions between disulfiram and intravenously administered cocaine before launching a large-scale phase III multicenter trial with this medication.

Cooperative Research and Development Agreement (CRADA) With NeuroSearch, AG

NIDA entered a CRADA with NeuroSearch, a small Danish company, to perform research on NeuroSearch's proprietary compound, NS2359, targeted exclusively as a potential treatment for cocaine addiction. As part of this agreement, NeuroSearch conducted the first human subject safety trials on this product. Assuming that analysis of the data generated from this trial is supportive, NIDA will undertake the next safety and/or efficacy trials indicated to further research and development of this compound.

The final protocol for this safety cocaine-interactions study has been approved by the site's institutional review board and the FDA. The study is under way and is scheduled to be completed in December 2002.

GBR 12909 (Vanoxerine)

Major neurochemical effects of cocaine include release of DA, serotonin, and noradrenaline via a transporter mediated exchange mechanism. There is considerable evidence that the initiation and continuation of cocaine use is associated with the effects of the drug on the dopaminergic, serotonergic, and noradrenergic modulation of CNS function. Animal studies suggest that the mesocorticolimbic dopaminergic pathways are important mediators of cocaine's reinforcing and addictive properties. Cocaine binds to these transporters and blocks removal of these neurotransmitters from the synaptic gap.

The neurobiological mechanisms underlying the effects of cocaine are not well understood. Preclinical studies indicate that cocaine's blockade of the DA transporter plays a key role in producing cocaine's addictive and reinforcing effects. Primate and nonprimate studies have shown that GBR 12909 has a strong affinity for the DA transporter. GBR 12909 is a high affinity, selective, and long-acting inhibitor of DA uptake that produces persistent and noncompetitive blockade of DA transporters and substantially reduces cocaine-induced increases in extracellular mesolimbic DA. In addition, GBR 12909 has a higher affinity than cocaine for the DA transporter. Ongoing research is seeking a DA-sparing cocaine antagonist that might be developed as a pharmacological treatment to block cocaine from acting at the transporter level to produce its reinforcing effects. GBR 12909 has been postulated to act by binding only to precise sites on the DA transporter that are required for cocaine binding and making the sites where DA binds to the transporter available.

A phase I clinical study was conducted in support of an investigational new drug (IND) application filed by NIDA. The main objectives of this study were to determine the safety, tolerance, and pharmacokinetics of multiple escalating dosages of oral GBR 12909 in healthy volunteers. In addition, positron emission tomography scans measuring the occupancy of the DA transporter by GBR 12909 were obtained. The occupancy scan results will be correlated with the safety data to determine an optimal oral dose of GBR 12909. The current study has completed all four dosage levels. Unexpected adverse effects include insomnia, increased libido, and disinhibition of aggressive feelings.

The study report from the phase I (healthy volunteer) study showed 30–40% DA transporter occupancy at the 100-mg dose level. Based on primate data showing equivalent occupancy levels at doses reducing cocaine self-administration, this may be clinically meaningful in cocaine treatment. Consultants reviewed the study in October 2001 and recommended a followup study in cocaine-dependent patients to address safety and other metabolic issues that were raised in the first study, in planning for a cocaine interaction study. The initial study in cocaine-dependent subjects is scheduled to start in 2002.

DA Agonists

The activation of the dopaminergic reward system in the brain appears to be the principal neurochemical mechanism involved in addiction to stimulants such as cocaine and amphetamine. Chronic abuse of these drugs results in DA deficiency in the brain, believed to lead to craving for stimulants, depression, anhedonia, and dysphoria.

Several clinical reports, such as those for amantadine, suggest that DA agonists may decrease cocaine use. NIDA proposes to clinically test several approved medications that would increase dopaminergic tone in the brain and to study novel compounds. Examples of these agents are direct DA agonists and partial agonists, DA precursors, reversible monoamine oxidase inhibitors, and drugs that inhibit DA, serotonin, and noradrenaline neuronal reuptake (mimicking cocaine but having a slower onset of action and, presumably, being less addictive).

Most recently, studies in rodents, and to a lesser extent in monkeys, have differentiated the roles of DA D_1 and D_3 receptors with regard to cocaine. The D_1 system may inhibit the effects of cocaine, whereas the D_3 system may provide a cocaine substitute of lesser dependence potential. Compounds that affect both systems are under study.

κ-Opioids

Recent studies have shown that κ-opioid compounds exhibit effects opposite that of cocaine in terms of DA release and neuron firing patterns. In animal studies, κ-opioids block drug discrimination and self-administration of cocaine and prevent context-independent sensitization to cocaine. NIDA and NIDA grantees are testing compounds of this class in clinical studies.

Glucocorticoid Antagonists

Studies have shown that cocaine causes the release of stress hormones known as glucocorticoids in both rats and humans. Some evidence from rat studies indicates that glucocorticoid antagonists and corticotropin-releasing factor (CRF) antagonists reduce self-administration of cocaine in a dose-related manner. NIDA will follow up on these basic research findings with additional studies aimed at developing a potential treatment for cocaine addiction. DTR&D is attempting to obtain CRF antagonist compounds from pharmaceutical company sources.

Immunology

In 1998, NIDA sponsored a meeting on the potential of use of peripheral blockers to prevent and treat cocaine addiction (for a summary, see www.nida.nih.gov/MeetSum/Peripheral/peripheral.html and www.nida.nih.gov/MeetSum/Peripheral/Index.html). The ability to block cocaine's entry into the brain or decrease its rate of entry (and thus attenuate the high produced) was discussed. Several approaches (e.g., active and passive immunization, catalytic antibodies) were actively explored. One of these theoretical constructs has now been translated into actual therapeutic entities that are at various stages of research and development, as listed below.

DTR&D-funded researchers reported that they have successfully immunized rats against many of cocaine's stimulant effects. Cocaine was prevented from entering the brain when rats were "vaccinated" with a substance that triggers the body to produce antibodies to cocaine. These antibodies then acted as biological sponges to which cocaine binds, thereby reducing the amount available in the blood to reach the brain. The results of this research are presented in "Suppression of Psychoactive Effects of Cocaine by Active Immunization" in *Nature,* December 14, 1995.

Researchers at The Scripps Research Institute demonstrated a greater than 70% reduction in cocaine uptake in the brains of rats inoculated with the antibody-producing compound compared with a group that was not inoculated. Researchers designed the compound so that the antibodies produced would respond specifically to the cocaine molecule yet not affect normal brain chemistry.

The researchers used an active immunization approach by developing a substance that, when administered to rats, would trigger the immune system to produce antibodies specific for the cocaine molecule. The researchers inoculated the rats over a 35-day period and then tested their responses to cocaine. The immunized animals showed significantly lower responses to the stimulant effects of cocaine than control animals because the immunization prevented much of the cocaine from getting to the brain. Cocaine concentrations in brain tissue of the immunized animals were found to be dramatically lower than those in brain tissue of controls.

Other immunotherapy research for drug abuse treatment has explored the use of catalytic antibodies and other external agents that can be used to treat cocaine dependence. The published research differs by inducing the production of antibodies that remain in the bloodstream for an extended period and block cocaine's effects after it is used.

The biotechnology company ImmuLogic Pharmaceutical Corporation (Waltham, Massachusetts) recently announced that they have devel

oped a cocaine vaccine. Barbara S. Fox of ImmuLogic discussed some of the company's findings in *Chemistry and Engineering News* (December 18, 1995) and at a January 1996 meeting of the Maryland Bioscience Alliance at the request of NIDA. ImmuLogic had previously received phase I SBIR funding from NIDA. In 1996, NIDA awarded a $700,000 SBIR award to ImmuLogic to complete preclinical development of a vaccine to treat cocaine dependence. Results of ImmuLogic's early vaccine work in animals have received attention in national and trade press, were presented at the College on Problems of Drug Dependence and were published in *Nature Medicine* (vol. 2 1996).

The vaccine links a protein to cocaine, resulting in a molecule that induces antibody formulation. Once titers reach a certain level, cocaine's ability to cross the blood-brain barrier is impeded. The award expedited completion of preclinical design and early testing of the vaccine. A phase I (dosing and tolerability) trial was successfully completed in 1999. ImmuLogic is no longer in business but sold the rights to the vaccine to Cantab Pharmaceuticals Research Ltd., a United Kingdom company specializing in vaccine development. NIDA has supported this research via a Strategic Program for Innovative Research on Cocaine Addiction Pharmacotherapy (SPIRCAP) and SBIR grant. Thirty-four subjects completed the initial phase I study in the United States. Specific antibody titers for cocaine were developed in the vaccine-challenged subjects. Under the SPIRCAP award, two additional studies are planned to convene this year: an inpatient study, examining the extent to which the antibody can block the effects of administered cocaine, and an outpatient study. To date, no adverse events have been reported, and the company plans to continue development of the vaccine. A phase II (safety and immunogenicity) study is planned to start in 2002.

Serotonin Antagonists

Cocaine increases extracellular levels of serotonin, DA, and noradrenaline in the brain. Serotonin is an extremely versatile neurotransmitter that activates numerous subreceptors. Studies of two subtypes of receptors (5HT-2 and 5HT-3) suggest that these structures may be involved in the rewarding and, possibly, mood-elevating effects of cocaine, directly and indirectly through an effect on DA. Antagonists of these receptors, which decrease DA release, may reduce cocaine craving and use. NIDA plans to test serotonin antagonists as potential medications for cocaine addiction.

Serotonin Reuptake Inhibitors

A major symptom of cocaine addiction is anhedonia, which is clinically similar to depression. It has been postulated that cocaine addiction may be a form of self-medication for chronic depression. Initial results of studies of the potent serotonin reuptake blocker fluoxetine (an antidepressant) have yielded mixed results in regard to being efficacious in facilitating abstinence from cocaine use. Evaluation of serotonin reuptake inhibitors as potential medications will continue. One such compound, the currently marketed antidepressant Venlafaxine, a serotonin and noradrenaline uptake inhibitor, is being studied by two NIDA grantees.

Nootropic Drugs and Cocaine Dependence

Abuse of stimulants, such as cocaine and amphetamines, is associated with some degree of neurological damage that results in cognitive impairment, brain perfusion deficit, stroke, intracranial hemorrhage, and development of early symptoms of movement disorders. NIDA-sponsored studies are under way to test several nootropic medications as potential treatments for cocaine-induced neurological deficits.

Cocaine "Receptor" Imaging Studies

In addition to the above categories of compounds being tested, a new and potentially useful technology is being investigated in regard to its value for predicting efficacy of potential cocaine treatment medications. Research in the field of structure-activity relationships has revealed highly selective and potent binding ligands for the DA transporter. NIDA intramural researchers have identified three generations of such compounds, with each succeeding generation being more selective and potent than the previous one. RTI-55, the first potent compound,

was shown to be an effective in vivo labeling agent in animal studies and was subsequently examined in human imaging studies by single-photon computed tomography. A second compound, RTI-121, was found to be more selective for the DA transporter but had a higher apparent lipid solubility and exhibited lower specific to nonspecific binding in vivo. NIDA researchers are testing new compounds and are using some older compounds (e.g., WIN-35,428) in brain imaging studies. Procedures have been developed for estimating the occupancy of transporter sites in vivo. DA transporter imaging studies of cocaine abusers have been completed (see section on GBR 12909). This technology may make estimating the effectiveness of a potential treatment compound or regimen possible by correlating receptor occupancy (as shown in imaging studies) with actual clinical results. NIDA will continue this line of research.

NIDA is also collaborating with NIMH, NINDS, and NIAAA to develop appropriate imaging ligands that will be essential to the study of many brain and CNS conditions, as well as the effects of various treatments.

Methamphetamine Treatment Discovery Efforts and Program Activities

Methamphetamine is a potent psychomotor stimulant that has seen periods of widespread use and abuse in the United States. Cocaine abuse and addiction surpassed methamphetamine use in the 1970s and 1980s, but methamphetamine abuse and addiction has resurfaced in some regions of the United States and is widespread in western U.S. cities such as San Francisco, Denver, Phoenix, and Los Angeles. According to the National Household Survey on Drug Abuse, an estimated 3.8 million people had tried methamphetamine in 1994, and the number had increased to 9.4 million by 1999. The epidemic is spreading to rural areas, and approximately 11,000 acute hospital admissions nationwide were related to amphetamine toxicity in 1999.

No accepted treatment medications exist for methamphetamine addiction or abuse. As a result, NIDA has developed a medication discovery program for methamphetamine and is funding several extramural and intramural studies to develop medications to treat methamphetamine abuse.

Preclinical Methamphetamine Program

Methamphetamine abuse has become a substantial drug problem in certain parts of the Southwest, and data suggest that its use is increasing and spreading to other parts of the United States. Methamphetamine is a powerful stimulant that shares some similarities with but also differs from cocaine. The Methamphetamine Addiction Treatment Think Tank (MATTT) meeting was held on January 10, 2000, during which consultants congregated to consider the direction of a methamphetamine treatment development program. Based on recommendations of these consultants, several types of methamphetamine-specific screening assays are being developed to evaluate and characterize compounds for their potential usefulness in treatment of methamphetamine dependence. Some of the recommended assays are similar to those used in the Cocaine Treatment Discovery Program; there will be substantial overlap between the two programs. Existing contract protocols are being used to test compounds for their interactions with DA transporters, but additional assays will be used to measure DA release in vitro—an effect of methamphetamine that is not shared by cocaine. In addition, behavioral assays are being set up to assess a compound's ability to block the locomotor stimulant and discriminative stimulus effects of methamphetamine and to determine effects on methamphetamine self-administration. In addition, assays to measure effects of potential treatment compounds on the cardiovascular system, both alone and in combination with methamphetamine, are being developed. Finally, methods for assessing the neurotoxic effects of methamphetamine are under development and may be useful in assessment of potential treatment medications.

Clinical Methamphetamine Program

To implement the recommendations of the MATTT meeting, a group of sites to conduct clinical trials for methamphetamine-dependent patients was established. Six sites were selected

where the epidemic is currently concentrated: two in the Midwest (Des Moines, Iowa and Kansas City, Kansas) and the other four in Los Angeles and San Diego, California, San Antonio, Texas, and Honolulu, Hawaii. A behavioral study was conducted in each of these sites as a pilot test of their capacity and readiness to engage this new field of clinical research. These sites proved ready to move into clinical testing of potential new pharmacotherapies for methamphetamine addiction treatment. Two phase I studies are under way: one at the University of California, Los Angeles (UCLA) for selegiline and the other at the University of Texas at San Antonio for bupropion for safety interactions with amphetamine. After these studies, and if safety is not an issue, selegiline and bupropion will be advanced to outpatient studies. The first phase II outpatient study for ondansetron will be initiated in 2002. Plans are under way to obtain lobeline from a pharmaceutical manufacturer to study its safety profile with amphetamine and ultimately advance it to outpatient studies.

Methamphetamine abuse and addiction is becoming an international problem. The Ministry of Public Health, Kingdom of Thailand, requested and received technical assistance from the NIDA MDP. Personnel from the MDP and UCLA have spent much time providing technical assistance to help the government of Thailand prepare an infrastructure capable of undertaking research studies of methamphetamine dependence and psychosis.

Phencyclidine (PCP) Treatment

A researcher at the University of Arkansas for Medical Sciences in Little Rock, is receiving NIDA funding to develop a new generation of monoclonal antibody-based medications to treat drug abuse (Immunotherapy for Drug Abuse, R01-DA07610, and Antibody-Based Therapy for Methamphetamine Abuse, R01-DA11560). Research is focused on treatments for methamphetamine, Ecstasy (methylenedioxymethamphetamine), and PCP abuse. These medications function as pharmacokinetic antagonists and are designed to reverse the effects of drug overdose and/or help blunt the reinforcing effects of drugs of abuse. Because of the unique pharmacological profile of these new medications, they would be well suited for use with other more conventional chemically based medications and treatments, such as behavior modification to aid in the long-term recovery from drug addiction.

If successful in humans, these treatments will not only provide a rapid reversal of drug effects in an emergency room setting, but they will also reduce or prevent the long-term medical problems associated with stimulant drugs of abuse (e.g., neurotoxicity and addiction). The studies of treating PCP effects are the most advanced and are providing the model system for development of antibody-based therapies for other classes of drugs and toxins. To this end, an anti-PCP monoclonal antibody that rapidly (in minutes) removes PCP from the CNS of rodents has successfully been developed. The group is focused on completing the preclinical animal studies necessary to file an IND application and on refining previously developed scale-up methodology for the production of the monoclonal antibody fragments. Other studies have shown that a single administration of an anti-PCP monoclonal IgG antibody can significantly reduce PCP's behavioral effects and CNS concentrations for at least 2 weeks, even when PCP is repeatedly administered in large doses to the animals over the entire 2-week period. This is important because a 2-week period in animals would be equivalent to about 1–2 months of protection in humans. The preliminary studies of antibody-based treatments for methamphetamine are showing the same type of long-term neuroprotective effects.

These researchers are collaborating with researchers at the University of Arkansas (Mechanisms of Onset and Offset of Rapid Stimulant Effects, K08-DA00339) to study the fundamental medical consequences of rapid input of drugs of abuse into the CNS. These experimental data from animal models of human drug abuse will help in understanding why drug abusers usually prefer rapid routes of drug administration (intravenous and smoking) over slower modes of administration (oral). Finally, the pharmacokinetic and pharmacodynamic studies are being used to help develop novel therapeutic approaches to the treatment of stimulant drugs of abuse.

National Institute of Environmental Health Sciences (NIEHS)

Overview

NIEHS supports basic research into the fundamental mechanisms of how environmental exposures interact with the human body to produce disease and dysfunction. This research on molecular pathways and environmental interaction has also yielded insights into the basic mechanisms involved in the pathogenesis of rare diseases and conditions.

Recent Scientific Advances

Maternal Serum Level of 1,1-Dichloro-2,2-Bis(*p*-Chlorophenyl)Ethylene and Risk of Cryptorchidism, Hypospadias, and Polythelia Among Male Offspring

Persistent halogenated organic pollutants (e.g., DDT, PCB, dioxin) command special attention in public health because they are ubiquitous in the ecosystem and may be causing adverse human health effects at background levels of exposure via diet. NIEHS is conducting several studies of the human health effects of background exposure to organochlorines. NIEHS scientists found that in utero DDE exposure was associated with a modest increase in cryptorchidism, hypospadias, and polythelia, although none of the results were statistically significant. Researchers found no association of in utero PCB exposure with any of the neurodevelopmental outcomes examined. The most notable finding so far has been that maternal pregnancy DDE levels were associated with increased risk of preterm birth and being born small for gestational age. Compared with boys whose mother had a serum DDE level of <15 mg/L (the lowest 20%), those whose mother had a serum level of ≥60 mg/L (the highest ~5%) had an adjusted odds ratio for preterm birth of 3.1 and an adjusted odds ratio for being small for gestational age of 2.6. Dose-response relations were present, and trend tests were statistically significant. The relation between DDE and small size persisted at 7 years of age, even after adjustment for birth size. Scientists found an association between PCB level and diabetes among pregnant women and an association between tetrachlorodibenzo-*p*-dioxin (TCDD) level and prevalence of type 2 diabetes.

Pathogenesis and Genetic/ Environmental Risk Factors for Autoimmune Diseases

Autoimmune diseases are often rare, incurable, costly diseases and appear to be increasing in prevalence in the population. These disorders are thought to result from chronic immune activation by selected environmental exposures in genetically susceptible individuals. The reasons for these reported increases in autoimmune diseases are unclear, although the increasing exposure to immune-altering foods, drugs, occupational exposures, and air and water contaminants may play a role. This project aims to uncover pathogenic mechanisms and environmental/genetic risk factors that lead to these diseases. NIEHS is focusing its studies on a group of orphan diseases—the adult and juvenile myositis syndromes, also known as the idiopathic inflammatory myopathies (IIMs)—as prototypic autoimmune diseases. Recent findings suggest different genetic risk factors for different ethnogeographic groups and a role for ultraviolet light exposure and maternal microchimeric cells in the pathogenesis of IIM in children.

Assessment and Therapy of IIMs

IIMs have an estimated incidence of 10 cases per million people per year, but they are associated with substantial morbidity and mortality. In many cases, treatment is unsatisfactory, and drug efficacy evaluation is hampered by the lack of reliable and standardized measures of disease outcome. No agents are currently licensed for treatment of IIM partly because of the lack of consensus on how to best measure disease in the myositis syndromes. A group of more than 70 specialists in adult and pediatric myositis, known as the International Myositis Outcome

Assessment Collaborative Study Group, is coordinating this project; the first workshop of this group was supported by ORD and NIEHS.

This project aims to develop and validate sensitive and efficient disease activity and damage-measurement tools, define improvement and deterioration criteria for use in clinical trials, and develop consensus on several clinical trial parameters. Achieving these goals should enhance the consistency by which clinical trials are performed, improve the capacity to compare different treatments, and encourage development of promising novel therapeutic agents for IIM.

Ataxia Telangiectasia (AT) Cancer

This research effort investigates the molecular mechanisms involved in cell-cycle checkpoint responses to exposures to ionizing radiation and other environmental agents in both normal human fibroblasts and fibroblasts that lack normal function of the AT cancer susceptibility gene products. In particular, researchers are interested in the role of the AT mutated (ATM) gene product in cell-cycle checkpoint responses to exposures to environmental carcinogens and the signaling pathways that are generated from broken DNA to the inactivation of cyclin/CDK protein kinase complexes. In addition to aiding the understanding of the process of carcinogenesis, these studies hold great potential for providing insight into the mechanism of action of non-genotoxic environmental carcinogens.

Progressive External Ophthalmoplegia (PEO)

NIEHS has found the first evidence of a mutator DNA polymerase causing PEO, a disease that causes paralysis of the eye and leads to further degeneration of other body tissues. PEO is an autosomal mitochondrial disease characterized by accumulation of point mutations and large deletions in mitochondrial DNA. Recently, the *Y955C* mutation in the human gene for DNA polymerase-γ *(polg)* was shown to cosegregate with autosomal dominant PEO. Polymerase-γ is the sole DNA polymerase in mitochondria and is responsible for mitochondrial DNA replication and repair. NIEHS researchers generated the *Y955C* mutation causing PEO in human polymerase-γ and analyzed the kinetics and fidelity of DNA synthesis by the purified mutant DNA polymerase. The *Y955C* mutation in the *polg* gene causes the DNA polymerase-γ to err when replicating mitochondrial DNA, leading to PEO. This analysis is the first example of a mutator DNA polymerase that generates the pathogenic mutations causing this human disease.

TCDD

NIEHS is studying the ability of chemicals found in the environment to initiate or exacerbate autoimmune disease. Specifically, NIEHS is examining postnatal immune consequences of prenatal exposure to TCDD, DES, and other endocrine-disrupting chemicals in several rodent models. To date, scientists have evaluated the potential for increased incidence and/or exacerbation of autoimmune disease in MRL/lpr autoimmune-prone mice exposed to either TCDD or mercuric chloride in utero.

MRL/lpr mice exhibit severe renal necrosis (glomerulonephritis) and increased levels of circulating autoantibodies similar to those observed in human systemic lupus erythematosus (SLE). The results suggest that developmental exposure to TCDD may accelerate the onset and increase the severity of SLE-like pathology in both male and female MRL mice. Furthermore, TCDD may be a stronger inducer of SLE than the prototypical control mercuric chloride in neonatal mice.

Treatment of Lead-Exposed Children (TLC)

Although TLC is now a cooperative agreement, NIEHS performed a clinical trial of succimer to prevent lead-induced developmental delay and behavior problems. Succimer lowered blood lead; however, when NIEHS tested the children at age 5 years, no differences were found in cognition or behavior. Scientists will test them again when they are 7 years old to determine whether differences emerge when they can be more thoroughly tested.

Li-Fraumeni Syndrome (LF)

Germline p53 mutations are associated with LF, a rare autosomal dominant disorder characterized by increased general cancer risk and tissue-specific effects. More than 80% of the inherited p53 mutations are missense changes in the DNA binding domain of the protein. Because p53 is a master regulator of diverse cellular responses to stress, primarily through transcriptional modulation of many genes, the extent or the specific functional impact of different p53 mutations may affect both the incidence and the tissue specificity of cancer progression in LF patients.

NIEHS developed a highly sensitive p53 functional assay in the yeast *Saccharomyces cerevisiae* to characterize p53 alleles that might retain partial transactivation function toward any of 30 p53 mammalian transcriptional response elements. Researchers found that wild-type p53 exhibits a wide spectrum of relative transactivation capacity.

A detailed p53 mutant functionality database of germline p53 alleles will be an important tool in characterizing subgroups of LF families. Knowledge of p53 functional changes is expected to increase understanding of tissue specificity in cancer development and help in treatment.

Friedreich Ataxia (FRDA)

FRDA is the most common cause of recessive ataxia, occurring in 1 in 30,000 whites. The yeast homolog of the *FRDA* gene, *YFH1*, which codes for the protein frataxin, is responsible for regulating the amount of iron in the mitochondria. The absence of frataxin leads to iron accumulation and production of radicals. NIEHS scientists have established that frataxin limitation leads to nuclear, as well as mitochondrial, DNA damage via reactive oxygen species, resulting in chromosome instability and mutations. This novel finding has implications for understanding the pathological symptoms associated with the disease, potential treatment strategies, origins of aging, cancer, and the role of mitochondria in nuclear changes.

Nijmegen Breakage Syndrome (NBS)

NBS is a rare autosomal recessive disorder characterized by increased sensitivity to ionizing radiation, defective cell-cycle checkpoint responses, and elevated cancer incidence. Both NBS and a related ataxia telangiectasia–like disorder are caused by mutations in the chromosomal DNA repair genes *hNBS1* and *hMRE11,* respectively. Functional homologs of the human genes, referred to as *XRS2* and *MRE11,* are present in the genetically tractable budding yeast *S. cerevisiae.* NIEHS and others have demonstrated that these genes perform similar functions in both yeast and human cells. The MRE11 and XRS2 proteins are components of a complex with DNA exo- and endonuclease activities. Recently, NIEHS researchers found that the nuclease function is critical in repairing a novel category of chromosome breaks (those ending in a hairpin cap) and have suggested that the complex may be the guardian of the genome against this category of breaks.

Research Initiatives

TTP and Related Proteins in Inflammatory Diseases

One major area of laboratory study began with the cloning of a gene that was rapidly induced by insulin. The protein encoded by this gene, TTP, is the prototype of a novel class of CCCH zinc finger proteins. TTP is rapidly induced, translocated from the nucleus to the cytosol, and phosphorylated on serine residues by insulin and many other mitogens and growth factors. Mice deficient in this protein develop a complex syndrome characterized by arthritis, wasting, dermatitis, and early death.

NIEHS scientists recently demonstrated that TTP deficiency in mice also leads to increased stability of the mRNA-encoding granulocyte-macrophage colony-stimulating factor (GM-CSF), a cytokine important for maintenance of the normal white blood cell count. Studies in cell-free systems and cultured cells are under way to identify inhibitors of this interaction, which might be useful therapies for neutropenic states. Concerning the mechanism of action of

TTP and its relatives, NIEHS reported that these proteins can destabilize certain mRNAs, even when those mRNAs do not contain polyA tails, indicating that initial deadenylation is not required for subsequent mRNA degradation. NIEHS began to establish the "rules" that govern the binding of this novel class of RNA-binding proteins to its target sequences and identified several protein-coding polymorphisms and one nonexpressing mutation in the human gene-encoding members of this family of proteins. Studies are under way to determine the biochemical and clinical significance of some of these variants.

Diet Effect on Occurrence of Chronic Disease

Diet may affect the risk of several chronic human diseases, although additional research is needed. A project under way has two main components: the study of diet-cancer relations and the study of diet in relation to risk of amyotrophic lateral sclerosis (ALS). Defects in antioxidant defenses (e.g., superoxide dismutase 1) are one cause of ALS; thus, it is reasonable to suspect that antioxidant intake may also affect the incidence or progression of this disease.

One research focus on diet in relation to ALS has been an analysis of dietary data from a case-control study of ALS. NIEHS examined the dietary intake of calcium, magnesium, and antioxidants among 107 ALS cases and 262 community control subjects. Overall, these dietary factors were not related to ALS risk, although modestly protective associations were suggested for magnesium and lycopene.

A second study of the same relationship is an add-on to a large cohort study under way at NCI. The cohort consists of approximately 600,000 members of the American Association of Retired Persons (AARP) who have completed a dietary questionnaire. Researchers expect approximately 150 cases of ALS to develop in this cohort.

Systemic Lupus Erythematosus (SLE)

SLE is a severe, disabling autoimmune disease. Approximately 90% of those affected are women. Although few studies provide detailed data pertaining to the prevalence of this disease, conservative estimates indicate that 100,000 women in the United States live with SLE. Researchers recently finished data collection in the Carolina Lupus Study, the largest population-based case-control study of hormonal and environmental risk factors for SLE development conducted to date. Four specific analyses based on these data were presented at the annual meeting of the American College of Rheumatology in 1999 and 2000. These analyses include hormonal and reproductive risk factors, medical history risk factors (e.g., allergies, infections), occupational silica dust exposure, and demographic differences in the clinical and immunological presentation of the disease.

Incontinentia Pigmenti (IP)

IP is a genetic disorder characterized by unusual patterns of discolored skin. Males with this disorder usually die before birth, so females are the major patient group. In rare cases, IP can cause developmental abnormalities such as dwarfism and clubfoot. NIEHS studies have definitively linked IP with deficiency of IKKγ/NEMO expression. This connection provides additional evidence for the importance of the IKK complex and NF-κB in prevention of programmed cell death in mice and in humans. IKKγ/NEMO-deficient mice can be used as a model for studying IP, which will help women with IP make more informed reproductive decisions.

Refsum Disease

NIEHS scientists are studying phytol metabolites, which are activators for the nuclear receptor RXR. Patients with Refsum disease accumulate the metabolite phytanic acid to levels about 100 times higher than normal. Because phytanic acid is alleged to solely originate as a result of diet, NIEHS researchers are examining the effects of removing it from the diets of rodents.

SGD Syndrome

Lactoferrin is an antibacterial and antiviral protein that is the major protein in the specific granules of the neutrophils. The only genetic disease linked to lactoferrin is SGD syndrome, in which patients lack the specific granules in neutrophils. SGD is characterized by lactoferrin deficiency with recurrent infections.

DNA Triplet-Repeat-Based Diseases

There are more than 14 rare neurological and neuromuscular diseases (including Haw River syndrome, which affects a small group of African American families in North Carolina) that result from the expansion of triplet-repeat DNA sequences. NIEHS scientists are investigating the underlying systems responsible for triplet-repeat expansion and have proposed a molecular model in which triplet expansion results from a deficiency of 5′-flap cleavage during DNA replication. In particular, the interaction of the human enzyme responsible for 5′-flap endonuclease cleavage *(FEN1)* with other components of DNA metabolism (e.g., proliferating cell nuclear antigen and DNA polymerases δ and ε) is being addressed genetically. Researchers have now established that the nuclease function of the replication protein DNA polymerase δ may also play an important role in processing replication intermediates. During lagging strand synthesis, a replication intermediate is created that must be processed by either DNA polymerase δ or the *FEN1* nuclease. The lack of processing is proposed to lead to a double-strand break that may be instrumental in triplet-repeat expansion. This research will further understanding of how the disease might arise and indicate possible consequences of variations in relevant DNA metabolic proteins.

Funded Studies

The following studies are funded by the Division of Extramural Research:

- Interactions of Environmental Toxicants With Leukocytes
  Johns Hopkins University
- DNA Crosslinking in Chromium Toxicity and Carcinogenesis
  George Washington University
- Molecular Biomarkers for Human Liver Cancer
  Johns Hopkins University
- Mechanism of Porphyria Caused by TCDD
  Dartmouth College
- Toxicology of Metal-Induced Immunopathology
  Wadsworth Center
- Lipid Peroxidation/Cell Signaling CC14-Induced Fibrosis
  University of Colorado Health Sciences Center
- Stachybotrys-Induced Hemorrhage in the Developing Lung
  Case Western Reserve University
- Survival Models for Mapping Genes for Complex Diseases
  University of California, Davis
- Genes and Chemical Exposure Associated With SLE Risk
  Center for Blood Research
- Community Outreach for CTD Screening in High-Risk Groups
  Brigham and Women's Hospital
- Perinatal Estrogen, Oxidative Damages, and Uterine Lesions
  University of Alabama at Birmingham
- Environmental Lead, Thyroid Function, and Neurodevelopment
  Columbia University Health Sciences
- Environmental Factors in the Etiology of Autism
  University of California, Davis
- Role of Somatic mtDNA Mutations in Neurodegeneration
  Beth Israel Deaconess Medical Center
- Community-Based Research of Autoimmune Disease and Asthma
  University of Buffalo, State University of New York

- Effects of Dietary Aluminum on Amyloidosis in a Transgenic Mouse Model
  University of Pennsylvania
- Hepatitis Virus, Alcohol Exposure, and Oxidative Stress
  University of Texas, MD Anderson Cancer Center
- A Translational Autism Program at the M.I.N.D. Institute
  University of California, Davis

Workshops and Conferences Cosponsored With ORD

Conference on Thyroid Hormones, Brain Development, and the Environment

Thyroid hormone (TH) is essential for normal brain development. Clinical syndromes of cretinism and congenital hypothyroidism provide ample evidence to support this statement. However, recent clinical and experimental research demonstrates that TH is important for fetal brain development. In fact, TH is important for brain development before the fetus is capable of producing its own TH. Therefore, any condition or factor that impairs maternal thyroid function or interferes with thyroid hormone action during fetal development can potentially interfere with normal brain development.

The goal of the conference is to bring together clinical and basic researchers that are central to the five research areas listed below. The premise is that important research focused on the intersection of these areas will be greatly enhanced by communication among the investigators. Consequently, this conference will help define areas of research required to address issues related to the environmental factors impacting thyroid hormone action during brain development and subsequent changes in brain function. The conference will bring together toxicologists, neurobiologists, neurobehavioral toxicologists, developmental biologists, developmental toxicologists, endocrinologists, pediatricians, and epidemiologists, as well as public health officials and public interest groups. The focus will be on the use of new technologies and approaches and increasing coordination and collaboration among the scientific disciplines. The specific rare diseases that will be covered are cretinism and congenital hypothyroidism.

International Epidemiology Association World Congress of Epidemiology

The focus on environmental exposures and women's health and the emphasis on state-of-the-art research connecting these two areas makes this particularly relevant for NIEHS. Examples of recent work in two rare autoimmune diseases, SLE and scleroderma, will be a centerpiece of this symposium.

Information Workshop on α_1-Antitrypsin Deficiency

α_1-Antitrypsin deficiency (α-1) is a serious hereditary disorder. Carriers of α-1 are also at risk. The most prevalent defective genes, *PiS* and *PiZ*, are found in all major geographic regions worldwide. Recent studies have also demonstrated the presence of other genes that can modify the expression of either *PiS* or *PiZ.* As a result, marked differences may occur in the health effects of either defective gene in different ethnic subgroups. A strong environmental component exists for α-1, and afflicted individuals can be markedly affected by exposure to environmental chemical and particulate pollutants. Particularly harmful is exposure to tobacco smoke.

α-1 is widely under- and misdiagnosed all over the world. Less than 6% of individuals hypothesized to have α-1 are diagnosed in the United States. α-1 can cause liver disease in children and adults and lung disease in adults. The lung disease is a form of chronic obstructive pulmonary disease (COPD), often misdiagnosed as asthma or typical smoking-related COPD that causes disability and early death.

Although many strides have been made in research and treatment, α-1 is a life-threatening genetic disorder that currently has no cure. However, because α-1 is a single-gene disorder,

a cure is likely to be found soon. Currently, affected individuals can alter the disease progression with simple lifestyle changes. This will require the development of new screening programs worldwide for early identification of carriers and homozygotes/heterozygotes for the *PiS* and *PiZ* genes.

Workshops for Development of Outcome Measures and Consensus on Design Issues for Myositis Clinical Trials

NIEHS is sponsoring the second of two workshops to facilitate clinical trials for myositis. The first workshop will be held in November 2002 inNovember 2002 in San Francisco and is supported by ORD, NINDS, and NIEHS. The second workshop will be partially supported via a NIAMS clinical trials planning grant. The primary goal of the first meeting is to use the core set of outcome measures, recently defined by a group of international specialists, to develop a myositis disease activity index and a preliminary definition of improvement for use in therapeutic trials of adult and juvenile myositis. The primary goals of the second meeting are to finalize the definition of clinical improvement and a myositis damage index and to develop consensus on issues related to the general conduct of clinical trials in myositis.

The meetings will bring together a diverse group of experts, including rheumatologists, neurologists, statisticians, and others, who will use Delphi and nominal group techniques to reach consensus on these issues with data from adult and pediatric myositis cases collected at NIH. Several papers summarizing the findings of these workshops are expected to be submitted for publication at the conclusion of these meetings.

Development of a disease activity index with responder criteria will greatly facilitate the conduct and interpretation of results for myositis clinical trials. A single efficacy measure will bring standardization to this field by decreasing the ambiguity associated with the presentation of multiple outcome assessments typically determined in myositis studies and by ensuring that uniform, comprehensive, and validated measures will be used in all future myositis clinical trials. The index will also improve the efficiency of future trials, thus potentially decreasing the sample size needed for myositis clinical trials, which is crucial for these rare diseases. Additionally, the index may increase the ability to detect efficacy for agents that are marginal in benefit. These measures may also be useful to practitioners in the routine clinical care of patients. Given the lack of standards and consensus on many aspects of clinical trial design in myositis, a session to address these issues is essential. Together, the outcomes from these workshops should be a strong and necessary foundation on which the first effective multicentered myositis clinical trials can be based.

Symposium on the National Children's Study (NCS)

Each year, the Society for Epidemiologic Research has, at its annual meeting of approximately 800 epidemiologists, several symposia that focus on topics of special interest. This year, a symposium is planned to discuss NCS. NCS is now being planned and developed as a consequence of the Children's Health Act of 2000.

The purpose of the NCS is to study the effects of environmental exposures, from before birth through adolescence, on more than 100,000 children and their families. Planning will take up to 5 years. The study will involve subject enrollment at multiple sites around the United States. The main reason for the large size of the study is to enable a prospective examination of risk factors for several rare diseases, including autism spectrum disorders, cerebral palsy, mental retardation, and birth defects. Two European investigators who are key personnel in the similar, recently launched large cohort studies will speak at the proposed symposium. A U.S. speaker will give an update on the NCS, and the last speaker would critique the NCS. Such a symposium will be strategic for the NCS because it will 1) help pique the academic interest and help prepare participants for work on planning committees and upcoming related grant proposals, 2) provide a forum for a "big picture" discussion of NCS, and 3) help investigators plan joint studies among the three cohorts to in

crease the power to study additional diseases that are too rare for investigation by a single cohort.

Cellular and Molecular Biology of Xenobiotic Transport at the Blood-Brain Barrier

To focus on the fundamental properties of the tissues and transporters that make up the blood-brain barrier, their roles in xenobiotic toxicity and protection and in pathophysiological regulation of barrier function will be examined. A better understanding of barrier function is important for virtually all neurological disorders because the barrier prevents entry of therapeutics into the central nervous system (chemotherapeutics, antivirals) and is itself compromised as a consequence of brain injury. This conference will be of interest to scientists in universities and pharmaceutical companies and to members of our intramural and extramural programs.

National Symposium Examining the Role of Social Environmental Factors in the Unequal Burden of Disease

NIEHS is interested in advancing science to elucidate underlying causes and mechanisms responsible for disparities in health. NIEHS, in collaboration with NIA, NIAMS, NHLBI, NIMH, the Office of Behavioral and Social Sciences Research (OBSSR), and National Institute for Occupational Safety and Help (NIOSH) issued a request for application, Health Disparities: Linking Biological and Behavioral Mechanisms With Social and Physical Environments. Twelve awards were initiated in FY 2000. This symposium will expand the scope of current research within this program and help define new directions. It can be coupled with the meeting of grantees in the health disparities program.

The purpose of this symposium is to address the state of the science and explore future opportunities in conducting research on the interaction between social environmental conditions, health status, and disease burden. Disparities in health between socioeconomically disadvantaged individuals and those who are more advantaged have existed for centuries. These health disparities may be defined as differences in disease incidence, mental illness, morbidity, and mortality that exist among specific populations. Disparities are most apparent and closely associated among populations with varying levels of socioeconomic status (SES). Significant evidence has demonstrated a gradient between SES and health status, with individuals of high SES having better overall health than those of low SES. Although the overall relationship of SES to mortality may attenuate with age, socioeconomic position continues to be linked to the prevalence of disability and chronic and degenerative diseases, including cardiovascular disease, many cancers, and neurodegenerative diseases. Low SES may result in poor physical and/or mental health by operating through various psychosocial mechanisms such as discrimination, social exclusion, prolonged and/or heightened stress, loss of sense of control, and low self-esteem. These psychosocial mechanisms may lead to physiological changes such as raised cortisol, altered blood pressure response, and decreased immunity that place individuals at risk for adverse health and functioning outcomes. Not only may SES affect health, but physical and mental health may have an impact on various measures of SES (e.g., education, income/wealth, and occupation) over the life course.

National Eye Institute (NEI)

Overview

NEI was created on August 16, 1968, by Public Law 90-489 to support and conduct research for improving the prevention, diagnosis, and treatment of diseases that affect the eye and vision. Eye diseases and blindness cost the nation an estimated $38.4 billion per year. More than 12 million people in the United States suffer some significant impairment of vision. Over the years, NEI-supported vision researchers have conducted many pioneering studies that have greatly advanced understanding of eye diseases, including those classified as rare, and provided eye care professionals with new tools and methods to prevent or cure many sight-threatening conditions. In June 1998, NEI published *Vision Research—A National Plan: 1999–2003.* This plan is the sixth in a series that dates back to the publication of *Vision Research Program Planning* in 1975. The development and publication of the aforementioned plans address the nation's visual health needs, including rare diseases of the eye and visual pathways.

Recent Scientific Advances

Leber's Congenital Amaurosis (LCA)

The genetic disorder LCA is one of several incurable forms of blindness collectively known as retinitis pigmentosa. It was first described in 1869 by Theodor Leber, who studied this condition in children less than 1 year old. No progress in understanding the cause of this childhood blindness was made for more than 100 years. Currently, no treatment exists for LCA or related early-onset retinal degenerative diseases. The Human Genome Project and the enormous progress made in the field of molecular genetics now offer hope that the disease may soon be treatable.

Mutations in LCA account for about 11% of patients with early-onset retinal degeneration. In 1994, NEI intramural scientists located an area on chromosome 1 that appeared to be involved in early retinal degeneration. This area contained the gene retinal pigment epithelium 65-kDa protein *(RPE65).* The scientists also showed that the *RPE65* gene product was located in the retinal pigment epithelium (RPE), a single layer of cells in close contact with the retinal photoreceptor outer segments. The function of the RPE65 protein was unknown, although it appeared to be involved in vitamin A metabolism in the retina. Assuming that a genetic defect in RPE might cause early retinal degeneration, the *RPE65* gene was used to screen for mutations in patients with LCA. This resulted in the identification of mutations that cause LCA when a child inherits two defective copies, one from each parent.

Research on LCA treatment has advanced enormously through recent studies of a naturally occurring congenital blindness in Briard dogs. In the course of long-term breeding by humans, these animals have acquired an *RPE65* mutation that is identical to one that causes about 20% of LCA cases in humans, although mutations in any of a dozen or so other genes are also known to cause LCA. Histopathological studies in homozygous dogs, which carry two defective copies of the gene, show abnormal rod photoreceptor cell morphology early in life, with slowly progressive photoreceptor degeneration and blindness.

Normal versions of the *RPE65* gene were genetically engineered into an adeno-associated virus vector. Thousands of copies were directly injected behind the retina, close to the RPE cells, of the right eye of three blind Briards who were between 2 and 4 months old. Ninety-five days after injection, the animals had vision in the treated right eye, judged by pupil response, electroretinogram, and behavioral testing. Tissue from a subretinally injected eye demonstrated persistence of the transferred DNA. The left (control) eye of these animals remained blind. All three dogs were seeing well 9 months after treatment with no ill effects.

Although previous studies had demonstrated that gene therapy could delay retinal degeneration, the current study demonstrates definitive recovery of function. The treated animals will continue to be studied to validate the effectiveness and safety of the treatment. Future studies will include application of gene therapy to both eyes of a dog.

Ocular Melanoma

Although rare, choroidal melanoma is the most common primary eye cancer in adults. Enucleation (removal of the eye) has been the standard treatment for choroidal melanoma for more than a century, because the melanoma cells can spread to other parts of the body and cause death. Radiation therapy emerged 20 years ago as a method to possibly preserve vision and reduce mortality. The Collaborative Ocular Melanoma Study was supported by NEI and NCI to compare enucleation versus radiation with respect to survival. Investigators previously reported that patients with tumors large enough to require removal of the eye who were randomized to either receive radiation treatment to the affected eye before it was removed or have the eye removed without radiation treatment showed similar 5-year survival rates of 60%. These researchers recently reported that patients with medium-size tumors who were randomized either to receive radiation therapy or to have the eye removed also had similar 5-year survival rates of 82% in the two groups. The results reveal that the size of the tumor is the most critical factor in influencing prognosis and emphasize the importance of early detection and treatment. With data showing similar survival rates for radiation therapy versus removal of the eye, quality of life issues become important factors in helping the patient and doctor determine treatment options.

Hallervorden-Spatz Syndrome (HSS)

HSS is a rare inherited neurological disorder associated with high accumulations of iron in the brain and that causes progressive degeneration of the retina and nervous system. In addition to a number of neurological symptoms that develop during childhood, some patients also develop degeneration of the retina. Death usually occurs in early adulthood, approximately 10 years after onset. Scientists recently discovered a defective gene that produces an ineffective enzyme in patients with HSS. The enzyme is needed by the body to use vitamin B_5, which is necessary to produce some of the body's essential compounds. These researchers hypothesize that the production of the ineffective enzyme by the defective gene causes blockage of normal metabolism and an accumulation of metabolic materials resulting from that blockage. It is believed that this accumulation results in degeneration of the retina and a high concentration of iron in the neural tissues. Research is now being focused on developing treatment strategies that bypass this defective enzyme, allowing the body to use vitamin B_5 to help make essential components. Understanding the biochemical defects in HSS may also provide insights into the effect iron has on other neurodegenerative diseases associated with high iron accumulations, such as Parkinson disease.

Ocular Albinism

Albinism includes a group of genetic disorders that share a reduction in retinal melanin pigmentation and significant visual abnormalities. Ocular albinism type 1 (OA1) is the most common form of ocular albinism; patients suffer from nystagmus, strabismus, foveal hypoplasia, photophobia, refractive errors, and decreased visual acuity, which can compromise vocational choice and quality of life. The more rare oculocutaneous albinism (OCA) is a heterogeneous group of congenital, mostly autosomal recessive but occasionally autosomal dominant disorders. This group includes the Angelman, Chediak-Higashi, Hermansky-Pudlak, Prader-Willi, and Waardenburg syndromes. OCA is characterized by a generalized disruption in melanin pigment synthesis in the hair, skin, irides, and eyeground.

OCA and OA1 have similar visual outcomes. The ocular manifestations of both OA1 and OCA include misrouting of RGC axons from the retina to the brain. Axons normally develop and grow along pathways by extending along adjacent axons and/or by detecting developmental cues. Various cues with chemoattractive or chemorepellent roles have been observed in the

developing visual system, where they control axon guidance and fiber crossing at the midline. A fundamental question in visual system development is how individual axons respond to these specific cues. Therefore, the abnormal axon routing seen in albinism presents an intriguing model system for developmental studies, particularly because the retinal disorganization is often associated with eye movement disorders and the development of myopia.

The relationship between misplaced axon projections and pigment deficits is a vexing and unresolved issue. The human OA1 gene product appears to be a 60-kD melanosomal protein. Point mutations in tyrosinase, the primary enzyme of melanogenesis, lead to both the absence of pigment in the RPE and the appearance of visual system axons that cross at the optic chiasm rather than remaining in the normal uncrossed position. What role pigment or its absence plays in these phenomena, or how pigment levels in the RPE affect the fate of RGCs on the opposite face of the retina, is unclear.

Corneal Dystrophies

Corneal dystrophies are a heterogeneous group of conditions that involve abnormal corneal development and result in defects in structure or clarity. The most common, though relatively rare, corneal dystrophy in the United States is keratoconus, characterized by a progressive thinning process of the cornea that may be accompanied by scarring. Keratoconus leads to progressive nearsightedness, astigmatism, and a cone-shaped cornea. Clinical care for keratoconus is time consuming for patients and doctors because of its chronic progression and difficulty in achieving a stable contact lens fit for visual rehabilitation.

Linkage analysis has shown that four clinical types of corneal dystrophy result from mutations in a single gene. Granular, Reis Bücklers, lattice type 1, and Avellino corneal dystrophies all map to the *βig-h3* gene, which encodes the keratoepithelin adhesion protein. In these four corneal dystrophies, the mutated keratoepithelin seems to form amyloidogenic intermediates that precipitate in the cornea, causing a progressive opacification. Three other corneal diseases also involve amyloidlike deposits: polymorphic amyloid degeneration, lattice corneal dystrophy type IIIA, and gelatinous droplike dystrophy. Keratoepithelin is a good candidate gene for further investigation in these families. Because of the accessibility of the cornea, these disorders represent excellent model systems for study of the molecular details of amyloid depositions in devastating illnesses such as Alzheimer disease.

Request for Applications (RFAs)

NEI has issued the RFA Ocular Albinism and the Neuroscience of Retinal Ganglion Cell Axon Guidance. This RFA will support studies of the basic mechanisms controlling axon guidance in the visual system and other visual phenomena related to the albino model. Studies of human tissue, genetic material, or subjects (including patients with ocular albinism and OCA are encouraged.

Program Activities

The National Advisory Eye Council and NEI have established the following goals for rare diseases research in *Vision Research—A National Plan: 1999–2003:*

- Identify novel causes of inherited retinal degenerations: further examine the cellular and molecular mechanisms whereby identified gene defects cause retinal degenerations.
- Further develop and critically evaluate therapies involving gene delivery, growth factors, and transplantation for treatment of retinal disease.
- Improve understanding of ocular surface physiology.

Workshops, Symposia, and Meetings

Craniofacial Muscle Specialization and Disease Workshop

The Craniofacial Muscle Specialization and Disease Workshop brought together scientific and

clinical experts from the United States and Europe. Leaders at the conference discussed craniofacial muscle embryology and developmental regulation, the intersection of structure with function for craniofacial muscle motor output, and the known biochemistry and pharmacology of these unique muscle classes. Craniofacial-selective or -resistant muscle disorders—myasthenia gravis, congenital fibrosis syndromes, and Duchenne muscular dystrophy, respectively—were discussed as models for understanding the underlying pathology of unique muscle types compared with more common muscular dystrophies. The following are recommendations that were promulgated from the workshop:

- Delineate the basic anatomy and ontogeny of the extraocular and craniofacial muscles.
- Promote research identifying aging-related changes in the structure and function of extraocular and craniofacial muscles.
- Determine the role of afferent feedback on normal muscle function.
- Develop and apply new research strategies and technology to the examination of craniofacial muscle biology. Molecular genetic approaches to muscle development and regeneration, the application of magnetic resonance imaging technology to visualize intact muscle, and gene replacement strategies to strengthen muscle are examples of important tools useful to advance the study of normal and diseased craniofacial muscle.

Encourage collaborative research across scientific disciplines to provide new insights into and knowledge about the functional biology of extraocular and craniofacial muscle systems and the pathogenesis of craniofacial-selective and -resistant diseases.

Ocular Toxoplasmosis Conference

NEI hosted a conference, with support from ORD, on ocular toxoplasmosis in which scientific and clinical experts from the United States, Africa, South America, and Europe participated. Conferees discussed molecular, epidemiological, and basic biology of this blinding eye disease. Several guidelines were developed for the diagnosis and clinical management of ocular toxoplasmosis. Additionally, an international work group was established to study the epidemiology of ocular toxoplasmosis in sub-Saharan Africa and Brazil. Basic biological studies will include genotyping *T. gondii* strains found in these regions.

National Institute of General Medical Sciences (NIGMS)

Introduction

NIGMS supports broad-based fundamental research that is not targeted to any specific organ system or disease. Examples include studies on the structure and function of organelles and membranes at the cellular and molecular level, investigation into the organization and function of the genome in organisms from bacteria to man, development of new and improved instrumentation and technology for application to biological problems, studies on basic biorelated organic chemistry for the elucidation of biosynthetic pathways and the development of new synthetic strategies for molecules of biological interest, and investigation into basic pharmacological mechanisms at levels ranging from the receptor to the molecular. In general, support of investigations related to specific diseases, unless of wide applicability across disease or organ system lines, is not the responsibility of NIGMS but rather would be assigned to one of the categorical Institutes.

Overview

Human Genetic Cell Repository

The NIGMS Human Genetic Cell Repository is a valuable resource for investigators studying genetic disorders. The repository, located at the Coriell Institute for Medical Research in Camden, New Jersey, collects, characterizes, maintains, and distributes cell lines from patients and families with a wide variety of genetic disorders and from healthy individuals whose tissues serve as controls. More than 6,600 cell lines representing more than 500 different diseases are available to qualified investigators. The repository stimulates research on rare diseases by providing access to cell lines, and DNA samples derived from these cell lines, that are otherwise not readily available. Cell lines from patients with rare diseases, such as xeroderma pigmentosum, ataxia telangiectasia, Huntington disease, cystic fibrosis, fragile X syndrome-linked mental retardation, Niemann-Pick disease, Nijmegen breakage syndrome, morbid obesity, glycogen storage disease, and Bloom syndrome, were among the cell lines requested most frequently in the past year.

Recent acquisitions include samples from patients with the following rare disorders: adrenoleukodystrophy, Alexander disease, ATP synthase deficiency, Bloom syndrome, Fanconi anemia, galactosemia, malignant melanoma, multiple endocrine neoplasia type 2, and osteogenesis imperfecta. These cell lines, as well as those previously acquired, are used for biochemical, cellular, and molecular studies to clarify the causes of genetic defects. The repository has a growing collection of cell lines in which the mutation has been characterized at the molecular level. These include samples from patients with adrenoleukodystrophy, Alexander disease, Bloom syndrome, galactosemia, and osteogenesis imperfecta, as well cell lines with recently characterized mutations from patients with cystinuria, familial dysautonomia, Emery-Dreifuss muscular dystrophy, Friedreich ataxia, and neuraminidase deficiency.

In addition, the repository supplies DNA isolated from two complete panels of well-characterized human-rodent somatic cell hybrids and from chromosome-specific somatic cell hybrid panels for nearly every human chromosome. The hybrids are a valuable resource for investigators interested in mapping disease-related genes, frequently the first step in characterizing disease etiology.

In cooperation with NHGRI, the repository houses the 450 cell lines (and DNA derived from them) that comprise the DNA Polymorphism Discovery Resource. These samples, which represent the genetic diversity of humans, will help researchers identify genes that are involved in the etiology of complex genetic diseases such as many cardiovascular disorders and cancers.

Recent Scientific Advances

Bloom and Werner Syndromes

Two human genetic diseases marked by increased genome instability and a predisposition to develop cancer, Bloom and Werner syndromes, are caused by defects in the *BLM* and *WRN* genes, respectively. Although the sequences of these two genes are related to that of the RecQ DNA helicase that functions in DNA recombination in bacteria, the functions of *BLM* and *WRN* have not been clear. Results from NIGMS-supported experiments with *Saccharomyces cerevisiae* that have mutated versions of the yeast gene for this helicase, *SGS1,* indicate that this gene plays a role in suppressing aberrant recombination between segments of DNA that have extended regions of imperfect homology (homeology). Recombination events between homologous sequences are a necessary part of the repair mechanism that brings together the DNA breaks that occasionally occur during the normal replication process. The joining of imperfectly matched pieces brought together by homologous pairing, however, is a major mechanism in the production of gross chromosomal rearrangements. In the specific experiments supported by NIGMS, a 20-fold increase in accumulation of gross chromosomal rearrangements was found in yeast carrying a mutation in the *SGS1* gene compared with wild-type yeast. These findings are consistent with the hypothesis that human *BLM* and *WRN* gene products normally act as DNA helicases that preferentially bind to and unwind heteroduplexes, which contain mismatched basepairs, before they mature into inappropriately recombined DNA molecules that appear as abnormalities.

Xeroderma Pigmentosum (XP) and Cancer

XP is a rare genetic disease associated with various symptoms, the most serious of which is a 1,000-fold increase in the incidence of melanomas and other skin cancers. People with XP have mutations that affect one of two proteins, XPB or XPD, both of which are involved in DNA repair. Regardless of which protein is affected, individuals with XP are severely deficient in their ability to repair damaged DNA. Because accumulation of mutations has long been associated with carcinogenesis, the increased incidence of skin cancer in people with XP is generally thought to be caused by their inability to repair DNA damage from sun exposure. However, people with trichothiodystrophy or Cockayne syndrome (CS), who are unable to repair DNA damage, have symptoms that are strikingly similar to those of people with XP, except that people with trichothiodystrophy or CS are not unusually susceptible to skin cancer. These observations suggest that the inability of people with XP to repair sun-damaged DNA is not solely responsible for their increased incidence of skin cancer. NIGMS-supported researchers have shown in cells from unaffected people that XPB and XPD interact with other proteins that regulate transcription of the *c-myc* gene. In contrast, in cells from XP-afflicted individuals, XPB and XPD do not interact with those proteins, so *c-myc* is not regulated correctly. Because overexpression of *c-myc* is associated with tumorigenesis, this suggests that the XP mutations' effect on transcription of *c-myc,* and, perhaps, other genes involved in tumorigenesis, is responsible for the increased incidence of skin cancer in people with XP. These observations provide insight into the genesis of skin cancer in the vast majority of people who do not have XP and suggest that the proteins with which XBP and XPD interact might be targets for therapeutic intervention to reduce the incidence of skin cancer in XP patients.

Sanfilippo Syndrome

Many congenital disorders are caused by defects in key metabolic enzymes. Sanfilippo syndrome, named for the physician who first described the condition in the mid-1960s, is one example. This syndrome, which is a lysosomal storage disease, affects babies born with a hereditary deficiency of some of the enzymes that break down certain natural polysaccharides during normal degradation of the body's connective tissues. The condition is rare, occurring only once per 25,000 births, but there is no cure. Symptoms, varying in severity according to the number of defective genes, progressively worsen until afflicted chil

dren can no longer talk or move; these children usually die in their teenage years.

Although some inherited disorders can be detected in newborns with DNA-based screening, these methods can be time consuming, invasive, and expensive, because a separate DNA sample must be prepared for each disease screen. Moreover, genetic tests account only for the presence or absence of a gene, not variability in the gene's expression or in the biological activity of its protein product. To date, so-called enzyme activity tests remain the gold standard for diagnosing inherited metabolic disorders that result from defects in several enzymes in certain metabolic pathways. Sanfilippo syndrome is one example of such a condition.

NIGMS-supported researchers have devised a new method to analyze a sample of skin cells for enzymatic defects present in children with Sanfilippo syndrome. Currently, deficiencies in any of six enzymes are known to lead to Sanfilippo syndrome; this new method can identify the deficiencies in four of the cases. The technique is fast, sensitive, and minimally invasive. Although the method was developed as a diagnostic tool for Sanfilippo syndrome, it has many potential applications for other metabolic disorders caused by enzyme defects.

National Heart, Lung, and Blood Institute (NHLBI)

Overview

NHLBI provides leadership for a national program in the causes, diagnosis, treatment, and prevention of diseases of the heart, blood vessels, lungs, and blood; in sleep disorders; and in the uses of blood and the management of blood resources. Through research in its own laboratories and through extramural research grants and contracts, it conducts and supports an integrated and coordinated program that includes basic investigations, clinical trials, epidemiological studies, and demonstration and education projects. Although the major part of the research supported by NHLBI addresses common conditions such as hypertension, coronary heart disease, and chronic obstructive pulmonary disease, a significant amount of research is devoted to rare diseases in children and adults.

Recent Scientific Advances

Heart and Vascular Diseases Program

Abetalipoproteinemia

Abetalipoproteinemia is a recessive disorder characterized by the absence of apoprotein B (apoB)-containing lipoproteins from plasma. Fat malabsorption is severe, and triglyceride accumulation occurs. Acanthocytosis, a rare condition in which most of the red blood cells (RBCs) have multiple spiny cytoplasmic projections, is common. Additional symptoms appear to be secondary to defects in the transport of vitamin E in blood. Genetic, biochemical, and metabolic approaches to studying various aspects of the disease were under way in four grants in FY 2001. The disorder appears to be related to abnormal processing of apoB due to an absence of the microsomal triglyceride transfer protein (MTP). Studies indicated that MTP is implicated in both apoB and triglyceride secretion. Cells lacking the ability to make MTP are unable to assemble and secrete apoB-containing lipoproteins. However, when MTP production is rectified (through appropriate transfection), apoB-containing lipoproteins are once more assembled and secreted.

Antiphospholipid Syndrome (APS)

APS is characterized by the presence of circulating autoantibodies to certain phospholipids. It is clinically manifested by recurrent blood clotting disorders, a history of fetal deaths, and autoimmune diseases such as thrombocytopenia. One NHLBI-supported study is engaged in efforts to develop more standardized immunoassays that will reliably detect individual antiphospholipid antibodies and is investigating the role of the syndrome in atherogenesis. Circulating antibodies to oxidized phospholipids, particularly cardiolipin, were found to correlate with the presence of isoprostanes (strong biomarkers for atherogenesis and a means of indicating the extent of atherosclerosis). Genes of autoantibodies cloned on the basis of their ability to bind to oxidized phospholipids have been found to play an important role in atherogenesis and to confer protection against certain bacterial infections.

Arrhythmogenic Right-Ventricular Dysplasia (ARVD)

ARVD is a family of rare cardiomyopathies that result in sudden cardiac death and heart rhythm disturbances including fibrillation. Most forms are believed to be caused by inheritance of autosomal dominant mutations in genes whose identities remain largely unknown but that clearly affect myocardial integrity. ARVD is characterized by marked, selective, right-ventricular dilation, myocardial cell death, and cell replacement with fat cells and fibrous tissue. Expression in gene carriers is variable, but the outcome is frequently lethal in those who display symptoms. NHLBI supports work on ARVD at its Baylor University Specialized Center of Research (SCOR) in Sudden Cardiac Death and sponsors a network of three separate groups, each supported by individual R01 awards, to investigate causes of familial forms of ARVD and genotype-phenotype relationships. In FY 2001, SCOR investigators identified a candidate gene

product for a neuroblastoma apoptosis-related RNA-binding protein that may correspond to a chromosomal mutation identified earlier as being common to patients with the congenital form of ARVD.

Bartter Syndrome

Bartter syndrome, a rare autosomal recessive disease, typically manifests itself through salt imbalance and low blood pressure. Research on Bartter syndrome is currently being pursued as part of the NHLBI SCOR program in Molecular Genetics of Hypertension. The discovery that a mutation in an ATP-sensitive K^+ channel can lead to Bartter syndrome establishes the genetic heterogeneity of the disease and demonstrates that this K^+ channel may be an important regulator of blood pressure, ion balance, and fluid balance.

β-Sitosterolemia

β-Sitosterolemia is a rare inborn error of metabolism characterized by increased absorption of dietary cholesterol and plant and shellfish sterols. β-Sitosterolemia patients have a markedly increased risk of premature cardiovascular disease. Effective treatment is not currently available, although a number of drugs are under development. NHLBI supports research into β-sitosterolemia through its intramural molecular diseases branch and its extramural grant programs. One NHLBI-supported investigator at the Medical University of South Carolina who is investigating the molecular mechanisms of cholesterol absorption and excretion in families with β-sitosterolemia has identified two separate defective genes. Additional research is identifying specific ATP-binding cassette gene (*ABCG*) sterol transporter protein mutations in affected families.

Brugada Syndrome

Brugada syndrome is a rare inherited disorder characterized by cardiac electrophysiological abnormalities—specifically, right bundle branch block and ST elevation in the precordial leads—and is associated with a high occurrence of sudden cardiac death. The condition is currently believed to be similar in cause and potential treatment to some forms of the long QT syndrome (LQTS). Both appear to be caused by mutations at different locations in the *SCN5A* cardiac muscle Na^+-channel gene and by resulting aberrations in depolarization and repolarization of these cells. One NHLBI-supported study demonstrated in FY 2001 that distinct mutations within a single residue of *SCN5A* can give rise to either Brugada syndrome (tyrosine to histidine mutation) or LQTS (tyrosine-to-cysteine mutation), adding evidence for a close relationship between these disorders.

Congenital Heart Disease

Congenital heart disease affects about 8 in 1,000 live-born infants (32,000 per year in the United States), making it the most common birth defect. Abnormal formation of the embryonic heart results in both structural and functional heart defects. It is an important cause of infant mortality, pediatric and adult morbidity, and shortened adult life expectancy. About one-third of affected infants and children require open-heart surgery or interventional cardiac catheterization to repair or ameliorate their defects. Approximately the same proportion has associated extracardiac anomalies, such as chromosomal abnormalities and syndromes involving other organ systems.

NHLBI has supported research in pediatric cardiovascular medicine since it first funded heart research grants in 1949. NHLBI-supported researchers have been instrumental in developing diagnostic imaging techniques, including fetal imaging; surgical techniques, including various operations and refinements in cardiopulmonary bypass; and medical therapies used today to ensure healthy survival for most affected children. They have also made significant contributions to the epidemiology of congenital heart disease and to understanding the molecular and genetic basis of normal and abnormal heart development.

A key finding from NHLBI-funded researchers this year was the identification of a new gene, *Bop,* that is the primary controller in a cascade of genetic events leading to the development of heart ventricles in mouse embryos. This finding

may eventually lead to understanding ventricular malformations in humans.

DiGeorge Syndrome

DiGeorge syndrome occurs with an estimated frequency of 1 in 4,000 live births. It is characterized by many abnormalities, including cardiac outflow tract anomalies, hypoplasia of the thymus and parathyroid glands, cleft palate, facial dysmorphogenesis, learning difficulties, and other neurodevelopmental deficits. It is usually sporadic but may be inherited, and it is caused by deletion of a segment of chromosome 22. The specific abnormal gene has not been identified. NHLBI supports both human and animal studies of DiGeorge syndrome through several grants, including two SCORs in Pediatric Cardiovascular Disease. NHLBI-funded researchers' finding that mice with chromosomal deletions similar to those found in humans with DiGeorge syndrome have deficits in learning and memory could lead to improved treatment for psychodevelopmental abnormalities in affected individuals. Such results support the need for a comprehensive therapeutic approach to children with DiGeorge syndrome, such as the team approach developed by a SCOR at the Children's Hospital of Philadelphia.

Doxorubicin Cardiomyopathy

The generic drug doxorubicin (Adriamycin) is a potent broad-spectrum antitumor agent effective in treating various cancers, including solid tumors and leukemia. Unfortunately, its clinical use is limited by dose-dependent cardiac side effects that lead to degenerative cardiomyopathy, congestive heart failure, and death. In addition, some adult patients who had been treated with this drug as children are now developing dilated cardiomyopathy. Endocardial biopsies from patients undergoing doxorubicin therapy reveal a disruption of myofibrils, impairment of microtubule assembly, and a swelling of the endoplasmic reticulum. Doxorubicin cardiotoxicity is also characterized by a dose-dependent decline in mitochondrial oxidative phosphorylation and a decrease in high-energy phosphate pools.

Several NHLBI-supported investigators have reported research advances in the past year. One investigator demonstrated that cardiac tissue from doxorubicin-treated rats expresses an increased tolerance for withstanding short periods of oxygen deprivation. This observation provides novel insight into the molecular regulation of compensatory responses that may underlie the adaptation phenomenon that has been widely described for other types of cardiac challenge. The same investigator observed a potential cardioprotective effect against doxorubicin-induced mitochondrial cardiomyopathy by carvedilol, a nonselective β-blocker with α_1-receptor-blocking (vasodilating) and antioxidant properties. This promising result opens exciting opportunities for supporting clinical trials of carvedilol as protection against the debilitating side effects of doxorubicin. This is particularly relevant because the class of drugs known as β-adrenergic-receptor antagonists, which includes carvedilol, is currently widely prescribed as safe and effective prophylactic treatment for many other cardiovascular disorders, including congestive heart failure. Adding doxorubicin-induced cardiomyopathy to the list of indications for carvedilol may prove highly effective in reducing the incidence and/or severity of cardiac failure that limits the clinical success currently achievable with doxorubicin.

Another investigator is examining explicit pathways through which reactive oxygen species are involved in doxorubicin-induced cardiomyopathy. She has discovered a marked inhibition of activity of the myocardial membrane-associated enzyme phospholipase A_2 by clinically relevant concentrations of doxorubicin. This novel observation suggests new means of doxorubicin action and has significant implications for elucidating the mechanisms underlying doxorubicin cardiotoxicity and pharmacological interventions to prevent it.

Dysbetalipoproteinemia

Dysbetalipoproteinemia is a rare disorder with a strong heritable component characterized by the presence of β-migrating very low density lipoprotein (VLDL). The disorder leads to the formation of characteristic yellow skin plaque (xanthomas) and predisposes to early ischemic heart disease and peripheral vascular disease.

Research into the genetics and biochemical events underlying the etiology and pathophysiology of the disease is under way in two NHLBI-supported studies. A mutant form of apoprotein E (apoE2) has been identified as the primary molecular defect in dysbetalipoproteinemia. Animal models synthesizing apoE variants are being created to facilitate basic research. In FY 2001, animals expressing human apoE2 were found to have significant increases in the apoE2 content of VLDL and intermediate-density lipoprotein. High levels (>10 mg/dl) of apoE2 are accompanied by higher levels of total cholesterol (threefold) and plasma triglycerides (sevenfold).

Familial Hypercholesterolemia (FH)

FH is an inherited autosomal dominant trait characterized by elevated concentrations of low-density lipoproteins (LDLs). Cholesterol derived from LDL is deposited in arteries and causes heart attacks and xanthoma lesions on tendons and skin. The defect in FH is a mutation in the gene specifying the receptor for plasma LDL. The receptors facilitate removal of LDL; when deficient or absent, the rate of LDL removal is low, resulting in an elevated LDL level. The homozygous form of FH is rare (1 in a million), but people who have it are highly prone to premature coronary heart disease. Several NHLBI grants support studies on the biochemistry, genetics, and potential treatment of the disease. A major program grant supports research on various aspects of regulating LDL receptors and cholesterol levels in the blood. Genetically manipulated animal models have been created specifically to study FH. Regulation of LDL-receptor activity and other lipoprotein receptors involved in disease progression is being elucidated. Development of apheresis methods for removing excess LDL from plasma is progressing, and testing of a combination of pharmacological agents is being planned.

Familial Hypertrophic Cardiomyopathy (FHCM)

FHCM is associated with myofibrillar disarray in the heart muscle that in turn leads to fibrosis and hypertrophy (enlargement of the heart). Although patients may remain asymptomatic for some time, shortness of breath, palpitations, and heart failure eventually emerge, and sudden death ensues. Some die during childhood, whereas others survive to their sixties or seventies. FHCM is associated with mutations in more than one protein, suggesting a heterogeneous group of disorders. During the past decade, scientists made significant progress in uncovering the genes associated with FHCM. It is known, for instance, that FHCM can be caused by many different mutations in the contractile proteins that comprise the heart wall. However, understanding who will die suddenly or whether certain factors, such as high blood pressure or extreme stress, will trigger sudden death remains elusive. NHLBI supports research on the genetic basis and mechanisms involved through several investigator-initiated grants and in two SCORs in Heart Failure. One SCOR program has demonstrated that simvastatin reverses cardiac hypertrophy and fibrosis in a rabbit model, and losartan reverses fibrosis in a mouse model. This is the first time any drug has been effective in an animal model of FHCM. Additionally, these investigators have preliminary findings that spironolactone is an equally effective treatment, indicating that angiotensin II is involved in fibrosis and hypertrophy formation. An investigator in the second SCOR program observed that the immunosuppressive drug cyclosporin A dramatically exacerbates the hypertrophic response in his mouse model of FHCM and that the Ca^{2+}-channel blocker diltiazem prevents this cyclosporin A-mediated response. He is currently assessing the effect of Ca^{2+}-channel blockers on the course of FHCM in mice.

Familial Hypobetalipoproteinemia (FHBL)

FHBL is an apparently autosomal dominant disorder of lipid metabolism characterized by very low levels of apoB-containing lipoprotein cholesterol. One NHLBI-supported project is using genetic, biochemical, and metabolic approaches to study various aspects of the disease. In FY 2001, information gained from newly identified families with FHBL enabled researchers to markedly narrow down the chromosome region containing the responsible genes. The most promising of the 60 genes in this narrower region are now being sequenced. Also, eight fami

lies have been identified that may have a new form of FHBL, because they have a susceptibility region near, but not in, the apoB gene on chromosome 2.

Infectious Myocarditis

Infectious myocarditis, which affects both children and adults, is an inflammation of the heart muscle that sometimes leads to progressive heart failure and the need for heart transplantation. NHLBI supports both human and animal studies of the disease. The infectious agent, coxsackievirus B3, is believed to be involved in many clinical cases of human myocarditis. One NHLBI-supported investigator is studying both susceptible and resistant strains of coxsackievirus B3 to determine the role of natural killer cells and cytokines—components of the innate immune system—in the pathology of myocarditis. The presence of interleukin-12 and interferon-γ cytokines in mice indicates that a Th1 immune response is taking place. In male mice, this response appears to be related to increased disease. Another investigator is looking at the pathogenesis of acute rheumatic fever, a consequence of group A streptococcal bacteria. Here, too, evidence supports a role for a Th1 response in the pathogenesis of the disease. Reovirus-induced myocarditis in mice provides an outstanding tool with which to investigate non-immune-mediated myocarditis. The reovirus (*reo* is an acronym for respiratory enteric orphan) is a naturally occurring virus that is believed to cause mild infections of the upper respiratory and gastrointestinal tracts of humans. Studies have shown that viral RNA synthesis in cardiac myocytes is a determinant of reovirus-induced myocarditis. Furthermore, genetic analysis of reoviruses that cause myocarditis has implicated several specific viral genes *(M1, L1,* and *L2).*

Klippel-Trenaunay-Weber Syndrome (KTWS)

KTWS is a very rare vascular deformation disease involving capillary, lymphatic, and venous channels. It usually manifests itself as cutaneous port-wine capillary malformations, varicose veins, and enlargement of soft tissues and bone in one limb. KTWS symptoms are usually present at birth, with 75 percent of patients having symptoms before age 10 years. A molecular approach to characterizing the gene(s) that contribute to KTWS is being taken in an NHLBI-supported study at the Cleveland Clinic Foundation. The investigator proposes that KTWS pathogenesis involves disruption of the key genes for vascular morphogenesis during embryonic development and has characterized a KTWS translocation involving chromosomes 5 and 11 and identified a novel vascular gene as the strong candidate gene for KTWS.

LQTS

LQTS is characterized clinically by a prolonged QT segment on an electrocardiograph and is associated with syncope, ventricular arrhythmias, and sudden cardiac death. This family of related diseases is believed to be caused by alterations in the cardiac cell action potential induced by mutations in at least six cardiac ion-channel genes.

NHLBI supports research on LQTS through a SCOR on Sudden Cardiac Death and through numerous individual grants that address the various molecular, clinical, and genetic bases of the condition. In FY 2001, an NHLBI-supported study identified mutations in the genes encoding the β-adrenergic receptors that may be associated with acquired LQTS, indicating a possible role for nonchannel proteins in contributing to the development of arrhythmias and sudden death.

Niemann-Pick Type C Disease (NPC)

NPC is an autosomal recessive lipid-storage disorder that is usually characterized by excessive accumulation of cholesterol in the liver, spleen, and other vital organs. Affected patients have cardiovascular disease, enlargement of the liver and spleen (hepatosplenomegaly), and severe progressive neurological dysfunction. Biochemical analyses of NPC cells suggest impairment in the intracellular transport of cholesterol to postlysosomal destinations. The gene deficiency in Niemann-Pick disease types A and B has been identified as sphingomyelinase. The gene deficiency in types C and D has been identified as the NPC-1 protein, but few clues re

garding its potential function have been derived. Two NHLBI grants and a subproject in a SCOR program support research to study the regulation of intracellular cholesterol movement that leads to cholesterol accumulation in NPC.

Cholesterol accumulation in NPC results from an imbalance in the flow of cholesterol among membrane compartments. Characterization of a putative cholesterol sensor in the plasma membrane that affects cholesterol trafficking into or out of cells is under way. The gene deficiency in NPC that encodes a cholesterol-binding protein has been identified. These new data will help fill a major gap in current understanding of cholesterol transport in the cell. Building on current knowledge of how cholesterol enters lysosomes and what happens after lipid reaches the endoplasmic reticulum, researchers now face the challenge of elucidating how cholesterol exits the lysosomes and enters the endoplasmic reticulum.

Smith-Lemli-Opitz Syndrome (SLOS)

SLOS is an inherited autosomal recessive disorder caused by a defect in the enzyme that catalyzes the last step in cholesterol biosynthesis. As a result, endogenous cholesterol synthesis is inadequate to meet biological demands for functions such as membrane structure and bile acid synthesis, and the precursor 7-dehydrocholesterol and its derivatives accumulate. Newborns with SLOS have a distinctive facial dysmorphism; suffer from multiple congenital anomalies including cleft palate, congenital heart disease, genitourinary abnormalities, and malformed limbs; and exhibit severe developmental delays, digestive difficulties, and behavior problems. SLOS is thought to account for many previously unexplained cases of mental retardation.

In FY 2001, NHLBI supported two investigator-initiated grants whose research foci are relevant to SLOS. One study is conducting basic research in sterol balance and lipid metabolism on 50 infants with SLOS. The study is also investigating the effectiveness of cholesterol-supplemented baby formula in ameliorating some of the behavioral and digestive symptoms of SLOS and the effectiveness of simvastatin therapy in lowering the plasma concentrations of toxic forms of abnormal cholesterol precursor compounds. Intermediate evaluation of progress indicates that infants tolerate the treatments well. The second grant, which ended in FY 2001, focused on basic analytical chemistry aspects of SLOS in the hope of developing an improved diagnostic test. Diagnostic and screening tests for SLOS are based on the presence of abnormally high levels of certain compounds from the sterol biosynthesis pathway that build up because of a lack of needed enzymes. Improved chemistry methods developed with support from this grant have led to improved separation of these compounds and more accurate determination of their concentrations in blood and other biological fluids, such as amniotic fluid.

Tangier Disease

Tangier disease is a rare syndrome characterized by a deficiency in high-density lipoprotein (HDL), mild hypertriglyceridemia, neurological abnormalities, and massive cholesterol ester deposits in various tissues, such as the tonsils. The disease is inherited as an autosomal codominant trait and appears to be caused by hypercatabolism rather than a defect in HDL synthesis. A member of the ATP-binding cassette (ABC) transporter family, human *ABCA1* (located on chromosome 9) has been identified as the defective gene. *ABCA1* is conceived as the gatekeeper for eliminating excess cholesterol from tissues and is therefore key in determining the amount of cholesterol accumulating in the artery wall. Research on the cell biology and biochemistry of human *ABCA1* and its role in the disease is under way in two NHLBI-supported studies. In FY 2001, these studies found that unsaturated fatty acids reduce macrophage *ABCA1* content by enhancing its degradation rate. In addition, *ABCA1* was shown to be responsible for the transport of α-tocopherol from cells.

The NHLBI intramural molecular diseases branch has also been actively studying Tangier disease for a number of years and announced five major findings in FY 2001:

1. The complete genomic sequence and the regulatory elements modulating gene

expression have been determined for the ABCA1 transporter.

2. ABCA1 transgenic mice have been developed to study the mechanisms involved in removing excess cholesterol from cells.
3. The ABCA1 transporter has been shown to recycle from the cell surface to a late endocytic compartment, establishing a new pathway for the transport of intracellular cholesterol to the cell membrane for removal by HDL.
4. The specific plasma apolipoproteins in HDL that act as acceptors for cholesterol removed from cells mediated by the ABCA1 transporter have been identified, and the molecular structural requirements to function as cholesterol acceptors have been elucidated.
5. Overexpression of the ABCA1 transporter in mice results in a marked decrease in diet-induced atherosclerosis, indicating that the development of drugs to upregulate the expression of the ABCA1 transporter may be a new approach in treating cardiovascular disease.

Lung Diseases Program

Advanced Sleep Phase Syndrome

Advanced sleep phase syndrome is a rare genetically based sleep disorder characterized by an early-evening onset of sleep, normal sleep duration, and spontaneous early awakening. NHLBI supports basic research to elucidate the neural pathways through which the biological clock mechanism regulates sleep, clinical research to elucidate genetic risk factors, and applied research on the role of the biological clock in disturbed sleep and alertness of shift workers, school-age children, and drowsy drivers.

α_1-Antitrypsin (AAT) Deficiency

AAT deficiency is an inherited deficiency of a circulating proteinase inhibitor that is manufactured primarily in the liver. Deficiency states (circulating serum AAT levels <0.6 mg/ml) are associated with emphysema, presumably from inadequate protection against enzymatic destruction by neutrophil elastase. Fifteen percent of the AAT-deficient population also develop liver disease. NHLBI funds various clinical and basic studies on AAT deficiency, including studies of the molecular mechanisms that impair AAT secretion, methods of gene therapy delivery, and how to increase the availability of defective but partially active AAT. NHLBI-supported investigators are defining the abnormalities and degradation pathways of the AAT protein, characterizing the inflammation that leads to disease in various AAT deficiency states, and evaluating the possibility of treating the disease with drugs that would enhance the release of partially active mutant protein from liver cells. A genetics study of families is seeking to identify other genes that may modify the nature and severity of the disease as expressed in different individuals. In addition to research that specifically focuses on AAT, NHLBI supports related studies that address the general causes of emphysema; the function, synthesis, secretion, and interaction of the enzymes that are inhibited by AAT; animal models of other enzyme-inhibitor deficiencies; gene regulation; gene therapy; cellular signaling, injury, and repair; and protein processing.

Asbestosis

Asbestosis, an occupational lung disease, is the interstitial pneumonitis and fibrosis caused by exposure to asbestos fibers. New findings have improved understanding of the role of genetic susceptibility in lung injury from asbestos. After asbestos exposure, lung fibroblasts are activated to grow and produce connective tissue. Certain inbred mice, the 129 mouse strain, do not develop asbestos-induced fibrogenesis, whereas other inbred strains do. Studies with growth factors suggest that the fiber deposition in the lungs of 129 mice is results from an intrinsic difference in the ability of the lung fibroblasts to respond to growth factors. It was also demonstrated that excess levels of transforming growth factor-β can induce fibrogenesis in the lungs of fibrogenic-resistant mice, providing a clue as to how individual growth factors may contribute to the development of fibroproliferative lung disease.

Bronchopulmonary Dysplasia (BPD)

BPD is a chronic lung disease characterized by disordered lung growth, specifically, changes in cell size and shape and fewer alveolar structures available for gas exchange. It affects at least 10,000 very low birth weight infants each year and is associated with neonatal intensive care costs as high as $60,000 per patient. Incidence of BPD has increased in recent years because of the increased survival of smaller premature infants. The NHLBI Collaborative Program for Research in BPD provides a well-characterized primate model for multidisciplinary exploration of the disease's etiology. NHLBI also supports two clinical trials on the role of nitric oxide in preventing and treating chronic lung disease in premature infants. A safety and dosage phase II clinical trial for intratracheal instillation of the anti-inflammatory uteroglobin CC10 in premature infants is nearing completion. In FY 2001, reduction of ventilatory injury with nasal continuous positive airway pressure was demonstrated in the preterm baboon model of BPD; histological examination revealed thin saccular walls with minimal fibroproliferation and improvements in internal alveolar surface area. In addition, the NHLBI intramural research program developed new technologies for prevention of nosocomial pneumonia and ventilator-induced injury that may reduce patient morbidity and mortality in the intensive care unit.

Churg-Strauss Syndrome

Churg-Strauss syndrome is a rare disorder that was first reported in the 1950s. It is characterized by formation and accumulation of an abnormally large number of certain white blood cells (eosinophils), inflammation of blood vessels (angiitis or vasculitis), and inflammatory nodular lesions (granulomatosis). Onset typically occurs between 15 and 70 years of age, and the disease affects both males and females. Patients with the syndrome are often affected by asthma. Churg-Strauss syndrome can be severely debilitating, even fatal if untreated, but patients usually respond well to corticosteroid treatment. More than 90 cases of Churg-Strauss syndrome have been reported in less than 2 years by physicians who had switched asthma patients from corticosteroid therapy to antileukotriene therapy. It is unclear whether the increased reports of Churg-Strauss are the result of an untoward effect of the antileukotriene therapy or a primary eosinophilic disease that had been clinically recognized and treated as asthma but was uncovered as Churg-Strauss once the corticosteroid therapy was withdrawn. NHLBI does not currently support research specifically investigating Churg-Strauss syndrome; however, it does support numerous investigator-initiated grants studying the basic mechanisms of asthma, including examination of the role of eosinophils. NHLBI also supports clinical studies of severe asthma and of medications used in asthma management, such as antileukotriene therapy. An NIH workshop report on the relationship of asthma therapy and Churg-Strauss syndrome was published in the *Journal of Allergy and Clinical Immunology* in FY 2001.

Congenital Central Hypoventilation Syndrome (CCHS)

CCHS, also known as Ondine's Curse, is a rare disorder characterized by normal breathing while awake but shallow breathing that is not effective in moving fresh air into the lungs during sleep. NHLBI supports a basic research program to elucidate the anatomical and physiological organization responsible for neural rhythm generation and translation into breathing. Research is focused on improving understanding of how breathing is regulated and the conditions under which reflexive generation of respiratory rhythm is abolished. Identification of the neuronal pathways producing respiratory rhythm and pattern are prerequisite to fully understand various respiratory sleep disorders such as CCHS. Recent findings obtained from overnight sleep studies indicate that CCHS is associated with a diminished sensitivity to levels of carbon dioxide in blood during non-rapid-eye-movement (non-REM) sleep. During REM sleep, other neural drives to breathe appear to supervene to enable adequate ventilation. Genetic and pathological studies of CCHS patients may enable identification of the genes or areas of the central nervous system involved in the syndrome and the abnormalities in ventilation.

Congenital Diaphragmatic Hernia (CDH)

CDH is a developmental disorder that occurs once in every 2,400 births. Often CDH occurs in isolated fashion, i.e., not associated with other life-threatening anomalies or chromosomal aberrations. Affected neonates usually die soon after birth because lung tissue compressed by the herniated viscera is inadequately developed and because hypoplasia of the pulmonary vascular bed leads to pulmonary hypertension or persistent fetal circulation syndrome. For infants who survive this disease, the cost of postnatal care can exceed $100,000. In June 1999, NHLBI awarded a grant for an investigator-initiated clinical trial to test the efficacy of an in utero surgical technique to correct lung hypoplasia compared with postnatal care in a group of human fetuses at 24–28 weeks gestation in whom the most severe form of congenital diaphragmatic hernia had been identified. Because the group assigned to postnatal care had an approximate mortality rate of 30%, rather than the expected 80%, a much larger sample size was deemed necessary. Investigators were unable to arrange for the necessary multisite collaborations, so enrollment had to be terminated in July 2001. The patients already enrolled in the trial continue to be followed.

Cystic Fibrosis (CF)

CF is a multisystem disease characterized by defective transport of chloride and sodium across the cell membrane. It is the nation's number one genetic cause of death among children and young adults. More than 25,000 Americans have CF, with an incidence of about 1 in 3,300 among whites. Ninety percent of people with CF die from pulmonary complications. The responsible gene, the CF transmembrane conductance regulator *(CFTR),* was identified in 1989. More than 800 mutations and DNA sequence variations identified in the *CFTR* gene contribute to the highly variable presentation and course of the disease. NHLBI supports a vigorous program of basic, clinical, and behavioral research focused on the etiology, pathophysiology, and treatment of the pulmonary manifestations of CF. The NHLBI program in Gene Therapy for Cystic Fibrosis and Other Heart, Lung, and Blood Diseases focuses on overcoming the many barriers to CF gene therapy, such as vector entry, persistence of expression, selective targeting to appropriate organ or cell, toxicity of the vector, and host immune response. The program also evaluates potential new pharmacological therapies. An example of a promising therapeutic strategy being investigated for CF is the screening of compounds that upregulate the chaperone proteins, many of which are also heat-shock proteins, maintain *CFTR* in its proper shape to function correctly. This is the first study to describe a potential therapeutic role for the nonessential fatty acid glutamine in CF.

Lack of understanding of the pathogenesis of CF airway disease partly reflects ignorance as to the physiology of the airway surface liquids (ASLs) that are vital for gas exchange and lung defense in the normal lung. The ultimate goal has been to understand the dysfunction in ASL physiology that leads to the chronic infections in CF so that rational therapies can be designed.

A major scientific advance over the past year has substantiated the importance of low ASL volume in the pathogenesis of CF airway epithelial disease, contributing to thickened mucus generated by ASL volume depletion and greater adherence of mucins to the surfaces of the CF airways. Based on these findings, studies are under way to develop therapeutic approaches to normalize ASL volume in CF. In FY 2001, the NHLBI intramural program reported that diagnostic approaches based on immunological detection of the *Pseudomonas aeruginosa* type III secretory apparatus and its associated cytotoxins provide evidence for early colonization and/or infection in children with CF.

Idiopathic Pulmonary Fibrosis (IPF)

IPF is a rare chronic lung disease of unknown cause affecting 3–30 individuals per 100,000. Individuals with IPF develop abnormal, excessive scarring in the lungs that can cause progressive shortness of breath and coughing. Available treatments, most commonly with corticosteroids in combination with other potent drugs and less commonly with lung transplantation, do little to prevent a relatively rapid death in most patients. NHLBI-supported research on IPF is examining

the molecular and cellular events that trigger the inflammation of alveoli seen in the early stages of the disease and that influence progression to the irreversible, fibrotic end stage. Three NHLBI intramural observational clinical research protocols focusing on the natural history and pathogenesis of the disease are open for enrollment of subjects with familial or nonfamilial forms of IPF. The protocols have established collaborations with extramural sites and are working with the Pulmonary Fibrosis Association and other patient-support organizations to recruit patients. In FY 2001, NHLBI intramural scientists found that aberrant transcriptional control in alveolar macrophages may be a contributing factor in the pathogenesis of IPF.

Lymphangioleiomyomatosis (LAM)

LAM is a rare lung disease that affects women, usually during their reproductive years. Symptoms develop as the result of proliferation of atypical, nonmalignant smooth muscle cells in the lungs. Diagnosis is usually made by lung biopsy. Common symptoms include shortness of breath, cough, and sometimes coughing up blood. Patients often develop spontaneous pneumothorax or chylous pleural effusion (collapse of the lung or collection of milky fluid around the lung). The clinical course of LAM is variable but usually slowly progressive, eventually resulting in death from respiratory failure. Although no treatment has been proved effective in halting or reversing LAM, lung transplantation is a valuable treatment for patients with end-stage lung disease. Some patients with tuberous sclerosis complex (TSC), a genetically transmitted disease, develop lung lesions identical to those seen in LAM. In some cases, the clinical distinction between TSC and LAM may be difficult.

NHLBI supports research on LAM in both its intramural and extramural programs. Intramurally, the Institute has established a research laboratory at the NIH Clinical Center to learn more about the cause and progression of LAM at the clinical, cellular, and molecular levels. Researchers are determining the characteristics of the unusual smooth muscle cells that damage the lungs of LAM patients. An important aspect of the research is learning how growth is regulated in these cells.

The NHLBI extramural program supports a national LAM patient registry that is coordinated by the Cleveland Clinic Foundation; ORWH cofunds the registry with NHLBI. Patients began enrolling in the registry in summer 1998. Enrollment closed in September 2001, with 253 recruited LAM patients. The registry helps manage the collection and distribution of LAM tissue for current LAM projects and serves as a repository of LAM tissue for future research.

Understanding the genetic mechanisms leading to smooth muscle proliferation in LAM and the relationship between LAM and TSC increased during FY 2001. It was previously reported that mutations in the *TSC2* gene could cause pulmonary LAM, but recently, researchers proved that the same types of mutations occur in an individual patient, based on cells taken from LAM lesions in the lungs and from kidney tumors (angiomyolipomas). The evidence suggests that cells in lung and the kidney have a common genetic origin. This discovery may lead to new diagnostic and therapeutic strategies for women with LAM. Also, the NHLBI intramural program reported that data from its ongoing study of the natural history of LAM have established clinical, pathological, physiological, and genetic criteria that define disease severity and progression.

Narcolepsy

Narcolepsy is a disabling sleep disorder affecting more than 100,000 people in the United States. It is characterized by excessive daytime sleepiness and rapid onset of deep (REM) sleep. Other symptoms include abnormalities of dreaming sleep, such as dreamlike hallucinations and transient periods of physical weakness or paralysis (cataplexy). Through programs such as the SCOR in Neurobiology of Sleep and Sleep Apnea, NHLBI supports research on the regulation of sleep and wakefulness, the regulation of muscle tone during sleep, and the genetic basis of narcolepsy in humans and animals. One new study finds that low cerebrospinal fluid levels of hypocretin, a neurochemical messenger linking

sleep with the regulation of muscle tone, are highly specific to narcolepsy and could potentially be used as a diagnostic procedure. Another study has determined that hypocretin is an excitatory chemical in brain regions regulating sleep.

Persistent Pulmonary Hypertension of the Newborn (PPHN)

PPHN affects approximately 1 in 1,250 live-born term infants. Because of inappropriate muscularization of fetal pulmonary vessels, the lung arteries of affected newborns fail to dilate after birth to allow for normal blood flow through the lungs. These infants are poorly oxygenated and require costly and prolonged medical care, including intubation of the airway, inhalation of 100% oxygen, mechanical ventilation, and, often, heart/lung bypass (extracorporeal membrane oxygenation). One NHLBI SCOR on the Pathobiology of Lung Development is focused on the unique vascular response of the neonate to injurious stimuli to identify the basic molecular mechanisms involved in development of the vasculature. This research may provide information for the treatment of hypertensive pulmonary disorders such as PPHN. In late1999, enrollment began for a clinical study to address maternal risk factors such as cigarette smoking and antenatal exposure to the nonsteroidal anti-inflammatory drugs aspirin and ibuprofen. Experimental evidence consistently suggests that maternal exposure to these agents plays a role in the etiology of the disorder. Buccal cell specimens are being collected and stored for future genetic analyses should a relationship be demonstrated.

Inhaled nitric oxide (NO) is an experimental therapy that offers promise for less invasive treatment of PPHN. Recent studies suggest a critical role for endogenous NO as a modulator of vasoactive mediator levels that determine pulmonary vascular tone and reactivity. Three isoforms of NO synthase (NOS) are known in mammals, and all are developmentally regulated in the fetal lung. Recent work with a premature baboon model of BPD has demonstrated a decline in two NO isoforms, nNOS and eNOS, during the genesis of chronic lung disease. Other investigators report biphasic release of NO in response to shear stress during development. These findings suggest that NO plays an important role during lung development.

Primary Ciliary Dyskinesia (PCD)

PCD, also known as Kartegener syndrome or immobile ciliary syndrome, is an inherited disease characterized by defects in the cilia lining the respiratory tract. The result is impaired ciliary function, reduced or absent mucous clearance, and susceptibility to chronic, recurrent respiratory infections, including sinusitis, bronchitis, pneumonia, and otitis media. The disease typically affects children ages 0–18, but the defect associated with it has a variable clinical impact on disease progression in adults as well. Many patients experience hearing loss, male infertility is common, and situs inversus (organs located on the opposite side from usual) occurs in approximately 50% of PCD patients. Clinical progression of the disease is variable, with lung transplantation required in severe cases. For most patients, aggressive measures to enhance clearance of mucus, prevent respiratory infections, and treat bacterial superinfections are recommended. Although the true incidence of the disease is unknown, it is estimated to be 1 in 32,000 or higher. Recent results of NHLBI-supported studies provide new insight into identification of the genetic basis of PCD. As part of an overall effort to gather sufficient numbers of patients for phenotypic and genetic studies, an international database containing detailed pedigrees, natural history information, and clinical and physiological data of well-characterized PCD patients and family members is being assembled. A selected cohort of patients with defects in the outer dynein arm of cilia, the most common form of PCD, were chosen for initial genetic studies. Identification of 6–10 genes for the vast majority of common PCD patients who have outer dynein arm defects could lead to genetic diagnostic testing, enabling more definitive identification and earlier diagnosis of PCD patients.

Primary Pulmonary Hypertension (PPH)

PPH is a rare progressive lung disorder characterized by a sustained elevation of pulmonary artery pressure. It is associated with structural

changes in the small pulmonary arteries and arterioles, resulting in resistance to blood flow. The process eventually leads to an enlarged, overworked right ventricle that is unable to pump enough blood to the lungs, resulting in heart failure and death usually within 3–5 years of diagnosis. Estimates of the incidence of PPH range from 1 to 2 per million, with women being predominantly affected. Approximately 6–10% of cases are familial PPH, a form inherited as an autosomal dominant trait. NHLBI supports basic research on the cellular and molecular events underlying the pathogenesis of PPH. The dominant themes of this research are 1) isolation and characterization of a familial PPH gene, 2) better understanding of the structural aspects of the disease that cause proliferative and obliterative changes in the vasculature, 3) identification of genetic factors that affect functional and structural changes in the vasculature, 4) development of preclinical markers, and 5) identification and evaluation of more effective treatments.

In November 2001, FDA approved a new drug, bosentan (Tracleer), for the treatment of PPH. Bosentan is the first oral treatment approved for PPH and the first in a new class of drugs, endothelin receptor antagonists, to be commercially available. Endothelin, a potent vasoconstrictor that also stimulates growth of vascular cells, is present in high concentrations in the bloodstream of patients with PPH. Results of a small (32-patient) double-blind, placebo-controlled study suggest that bosentan, which acts by blocking endothelin receptors, increases exercise capacity and improves heart function in patients with pulmonary hypertension. Future trials should help clinicians better define the place of this new class of agents in the therapy of pulmonary hypertension. Larger studies are needed to address important issues such as improvement in survival and their potential use in severely ill patients who are receiving prostacyclin therapy. Other recent work suggests that levels of circulating endothelin may serve as prognostic markers for patients with PPH and as a tool in selection of patients who may benefit from treatment with endothelin receptor antagonists.

Multiple agents represent another approach beginning to be applied to the treatment of PPH. Investigators are exploring, for example, the use of an oral phosphodiesterase inhibitor (sildenafil) as a therapeutic adjunct to inhaled iloprost.

In very preliminary studies, sildenafil caused a long-lasting reduction in pulmonary artery pressure and pulmonary vascular resistance, with further improvement after iloprost inhalation. Similarly, a small pilot study of iloprost inhalation combined with epoprostenol treatment in patients who had adverse effects during treatment with epoprostenol showed that the combination therapy significantly reduces pulmonary artery pressure and improves cardiac index and other indicators of cardiopulmonary function. These findings suggest that combined therapies may be useful in improving treatment of PPH.

The discovery last year of a genetic cause of PPH has increased opportunities for research into the etiology and pathogenesis of PPH. New findings this year suggest considerable heterogeneity in mutations of a gene (bone morphogenetic protein receptor 2, *BMPR2)* associated with PPH. Additional genetic and/or environmental factors may be required to develop the clinical phenotype. Other PPH genes remain to be identified because about half of the PPH families have *BMPR2* mutations. Data suggest that many cases of apparently sporadic PPH may in fact be familial, because failure to detect familial PPH is complicated by incomplete expression within families, skipped generations, and insufficient family pedigrees.

Recent findings on the pathogenesis of PPH indicate that endothelial cells within plexiform lesions of patients with PPH have genetic alterations associated with genetic (microsatellite) instability and abnormal growth and gene expression similar to that seen in neoplasia. Other studies suggest that the disorganized growth of endothelial cells in plexiform lesions from PPH patients involves disordered angiogenesis, thus allowing endothelial cells to expand. Pulmonary smooth muscle cells from patients with PPH have recently been reported to show abnormal responses to cell growth-signaling pathways, and a recently published study from researchers in France found a link between abnormal ex

pression of a serotonin transport protein and abnormal proliferation of vascular smooth muscle in PPH patients.

Sarcoidosis

Sarcoidosis is a disease involving organ systems throughout the body in which normal tissue is invaded by pockets of inflammatory cells (granuloma). Most sarcoidosis patients have granuloma in their lungs. The disease can exist in a mild form that spontaneously disappears or in a severe form that results in a lifelong condition. The number of Americans afflicted is estimated to range from 13,000 to 134,000, with between 2,600 and 27,000 new cases appearing each year. As many as 5% of individuals with pulmonary sarcoidosis die of causes directly related to the disease. Morbidity associated with this disease can be severe and result in significant loss of function and decrease in quality of life. The causes of sarcoidosis are unknown, but disease development is thought to involve the victim's immune system. NHLBI supports laboratory-based research to investigate granuloma formation and obtain better understanding of initiating events, the disease process, and the contribution of susceptibility genes.

A multicenter NHLBI study conducted from 1996 to 1999 found that sarcoidosis patients were almost five times more likely than control subjects to report a sibling or parent with a history of sarcoidosis. Whites who were affected were much more likely to have an affected relative than were African Americans. However, investigators found that even for family members (siblings and parents), the risk of sarcoidosis is small (about 1%) and concluded that increased surveillance is probably not warranted. They also found that sarcoidosis appears to increase the risk of depression.

Blood Diseases and Resources Programs

Aplastic Anemia (AA) and Paroxysmal Nocturnal Hemoglobinuria (PNH)

AA is a form of bone marrow failure in which fat replaces hematopoietic cells, resulting in low blood count. In PNH, a clone derived from a single hematopoietic stem cell expands, leading to marrow failure, RBC destruction, and venous thrombosis. The NHLBI intramural hematology branch has a large clinical and laboratory program devoted to bone marrow failure syndromes, including AA and PNH. Bench studies include immunology, cell biology, virology, and molecular biology approaches to the failure to produce blood cells. Clinical studies include therapeutic interventions to reduce autoimmunity in AA patients. In FY 2001, the branch established an animal model of immune-mediated AA, showing the pivotal role of type 1 cytokines in severe marrow cell destruction. In addition, analysis of its large trial of immunosuppression in severe AA found that early robust improvement in blood counts is highly predictive of long-term survival without malignant evolution.

Cooley's Anemia

Cooley's anemia (also called β-thalassemia, thalassemia major, or Mediterranean anemia) is a genetic blood disease that results in inadequate production of hemoglobin. Individuals affected with Cooley's anemia require frequent and lifelong blood transfusions. Because the body cannot eliminate iron naturally, the iron contained in transfused RBCs builds up over many years and eventually becomes toxic to tissues and organ systems. In addition, many affected children acquire diseases such as hepatitis through years of transfusion exposure.

NHLBI's extramural research efforts related to Cooley's anemia include 1) identification of mutations in the globin gene cluster that lead to the disorder, 2) elucidation of the mechanisms and therapeutic approaches associated with naturally occurring mutations that result in elevated levels of fetal hemoglobin (HbF) in adult RBCs, 3) iron chelation, 4) clinically useful therapies and drugs such as gene therapy, 5) efficient identification and targeting of hematopoietic stem cells, 6) how ex vivo manipulation of stem cells alters their biological properties, and 7) improved vectors for use in gene transfer efforts. The Institute's strategic approach also includes a clinical research network to test new therapies and a program of sibling donor cord blood

banking and transplantation for hemoglobinopathy families.

FY 2001 witnessed a number of important scientific advances for Cooley's anemia treatment. New methods of transfusion therapy are being developed to improve adherence with deferoxamine regimens for patients receiving chronic transfusions. Less toxic methods of stem cell transplantation are being developed that may be useful for patients with thalassemia. For example, the NHLBI intramural program is working on a vaccine to prevent cytomegalovirus reactivation after stem cell transplantation via CMV pp65 protein canarypox construct. Finally, several compounds that increase HbF values have been described, including hydroxyurea, which is a compound in routine use in sickle cell disease (SCD); numerous butyrate-based compounds; and 5-azacytidine.

Creutzfeldt-Jakob Disease (CJD)

CJD is a slowly degenerative, invariably fatal rare disease of the central nervous system characterized by motor dysfunction, progressive dementia, and vacuolar degeneration of the brain. The disease has been associated with a transmissible agent. A protease-resistant protein (prion) is the hallmark of the transmissible spongiform encephalopathy (TSE) family of diseases to which CJD belongs. Classic CJD occurs worldwide at a rate of 1–2 cases per million per year. The lack of a rapid, sensitive, and specific test for TSE infectivity has slowed progress in the study and control of CJD and other prion diseases. Development of assay systems to detect prion diseases is a high priority for public heath. Assay systems could form the basis of a blood/tissue donor screening test and provide a diagnostic test for neurologists; currently, there is no way to detect the disease in its preclinical stage. These assays could also be useful in testing for TSE in animals, especially domestic animals used for human consumption. In FY 2001, NHLBI-supported investigators reported that mouse skeletal muscle can propagate prions and accumulate substantial titers of them. Because significant dietary exposure to prions might occur through the consumption of meat, even if it is largely free of neural and lymphatic tissue, a comprehensive effort to map the distribution of prions in the muscle of infected livestock is needed. Furthermore, muscle may provide a readily biopsied tissue that can be used to diagnose prion disease in asymptomatic animals and even humans.

Fanconi Anemia (FA)

FA is an autosomal recessive bone marrow failure syndrome characterized by a decrease in blood cells and platelets (pancytopenia), developmental defects, and susceptibility to cancer. Many FA patients can be identified at birth because of congenital anomalies, although approximately 25% do not have birth defects. FA is a clinically heterogeneous disorder that can currently be divided into at least eight different complementation groups designated A–G. Delineation of the interrelationship between FA proteins and their functions through localization and functional studies is one of NHLBI's high priority research areas. In addition, NHLBI supports research that focuses on identifying and cloning the remaining FA genes, developing protocols for efficient identification and targeting of hematopoietic stem cells, obtaining information on how ex vivo manipulation of stem cells alters their biological properties, producing improved vectors, and exploring the utility of cord blood banking. Two FA genes, *FAC* and *FAA,* that account for an estimated 75 percent of all FA patients world wide have been cloned. The cellular localization of the functional complex and the role of the complex in DNA repair and prevention of mutagenesis have been exciting developments over the past year. Recent transplantation protocols with fludarabine have renewed hope that stem cell transplantation may be a therapeutic option for FA patients.

Graft Versus Host Disease (GVHD)

Acute GVHD is a condition that typically occurs within 3 months after allogeneic hematopoietic stem cell transplantation. Donor T cells react against foreign tissue antigens in the recipient. GVHD is characterized by skin rash, liver dysfunction, vomiting, and diarrhea. Acute GVHD often precedes development of chronic GVHD, which may require years of immunosuppressive drug treatment. NHLBI supports basic and clini

cal research studies focused on understanding the pathophysiology of GVHD, especially in unrelated transplants. The NHLBI program emphasizes understanding the roles of both major and minor histocompatibility antigens in disease pathogenesis, development of tolerance, function of donor T cells in allogeneic hosts, and mechanisms of GVHD prevention, including depletion of donor T cells from the graft. Current studies attack the problem of GVHD from several directions: the variables that affect its induction and severity, the effector mechanisms, and whether GVHD can be suppressed while other necessary immune responses are maintained. The program supports two multicenter clinical studies: the Unrelated-Donor Marrow Transplant Trial of T-Cell Depletion and the Cord Blood Banking and Transplantation Study. The Blood and Marrow Clinical Transplant Network was funded in FY 2001 to conduct phase III trials, including studies of GVHD. To date, older or sicker patients have been excluded from allogeneic hematopoietic cell transplantation (HCT) because of toxicities from the treatment regimen. Recently, investigators have developed a less toxic regimen, based on the use of postgrafting immunosuppression to control graft rejection and GVHD, that has dramatically reduced the acute toxicities of allografting. Now HCT with the induction of potent graft-versus-tumor effects can be performed in previously ineligible patients, largely in an outpatient setting. In FY 2001, the NHLBI intramural research program described how the alloimmune environment reshapes the immune response of the donor after stem cell transplantation by identifying innate T-cell responses to known and putative tumor-specific antigens.

Hemophilia

Hemophilia is a hereditary bleeding disorder that results from a deficiency in either blood coagulation factor VIII or factor IX. About 20,000 hemophiliacs live in the United States, all of whom depend on lifelong treatment to control periodic bleeding episodes. NHLBI supports a broad spectrum of activities on blood coagulation and its disorders. Research on hemophilia includes viral and nonviral approaches for gene therapy, mechanisms of antibody inhibitor formation, modification of factors for improved therapeutics, safety of plasma-derived products, and blood product-associated infections. In addition, NHLBI supports basic genetic, molecular biology, and protein biochemistry studies of factors VIII and IX to increase understanding of their mechanisms of action and regulation. One program project investigates multiple approaches to developing gene-based therapies for hemophilia A and B; another studies new therapies that can be used in the presence of inhibitory antibodies.

Gene therapy studies by NHLBI-supported scientists have shown sustained expression of factor IX in mice and hemophilic dogs after muscle injection or intraportal administration of adeno-associated viral (AAV) vector expressing factor IX. Based on preclinical safety and efficacy data, a clinical study for intrahepatic delivery of AAV vector expressing factor IX was initiated in August 2001. Studies by NHLBI-supported investigators have increased understanding of the immune response to factor VIII that leads to the formation of inhibitory antibodies. Studies in a hemophilia mouse model demonstrated in FY 2001 that long-term immune tolerance to factor VIII could be induced after early blockade of the interaction between the antibodies CD40 and CD40L. In addition, the structure of a region of factor VIII bound to an inhibitory antibody was determined that could be useful in efforts to develop less antigenic factor VIII products.

Immune Thrombocytopenic Purpura (ITP)

ITP is an autoimmune disease manifested by production of antibodies that react with specific proteins on the surface of platelets, resulting in rapid clearance or destruction of platelets (thrombocytopenia) and clinically significant bleeding. The underlying cause is unknown, but the disease is associated with other autoimmune disorders. Although ITP may occur at any age, acute (temporary) thrombocytopenic purpura is most commonly seen in young children. About 85% of affected children recover within 1 year and experience no recurrence. Thrombocytopenic purpura is considered chronic when it lasts more than 6 months. Its onset may occur at any age. Adults usually have the chronic disorder,

and females are affected two to three times more often than males. Most adult patients respond at least transiently to standard therapies such as steroids and splenectomy, but most eventually relapse, and some develop severe chronic refractory ITP.

Part of the NHLBI research program on thrombosis and hemostasis is directed toward understanding the biology of platelet production from megakaryocytes, the function of the growth factor thrombopoietin (TPO), and the structure and function of platelet surface glycoprotein antigens. Studies on TPO have not borne out the initial promise of this therapeutic strategy. Whereas mice with the TPO gene knocked out maintained a basal level of circulating platelets and did not bleed, numerous human subjects with thrombocytopenia who received TPO developed antibodies to the protein, and their clinical conditions worsened. The investigators concluded that TPO is an amplification factor, but it may not be essential for megakaryocytopoiesis and platelet production. On the other hand, migration of the bone marrow megakaryocytes to a more permissible environment for platelet production could be critical. In another development, a monoclonal antibody, rituximab, directed to B-lymphocytes for the treatment of cancer, was found in initial studies to be beneficial for patients with ITP.

Lymphedema

Lymphedema is an accumulation of lymphatic fluid in interstitial tissue that causes swelling, most often in the arm(s) and/or leg(s) and occasionally in other parts of the body. Lymphedema can develop when lymphatic vessels are missing or impaired (primary or congenital) or when lymph vessels are damaged or lymph nodes removed (secondary). When the impairment becomes so great that the lymphatic fluid exceeds the lymphatic transport capacity, an abnormal amount of protein-rich fluid collects in the tissues of affected areas. Left untreated, this stagnant, protein-rich fluid not only causes tissue channels to increase in size and number, it reduces oxygen availability in the transport system, interferes with wound healing, and provides a culture medium for bacteria that can result in a lymphangitis infection. The incidence of primary lymphedema has been estimated to be between 1 in 6,000 and 1 in 300 live births, so it may be a rare disease or it may be a more common disease that predisposes to the secondary type and is underrecognized. NHLBI investigator-initiated projects are seeking to identify the developmental, molecular, and cellular defects that contribute to lymphedema and to design effective therapeutic interventions to treat both primary and secondary lymphedemas. In December 2000, NHLBI issued a program announcement (PA) inviting applications to study the pathogenesis and treatment of lymphedema.

Sickle Cell Disease (SCD)

SCD is an inherited blood disorder that is most common among people whose ancestors come from Africa, the Middle East, the Mediterranean basin, or India. SCD is the most common genetic blood disorder in the United States, affecting approximately 1 in 500 African American newborns and 1 in 1,000 Hispanic newborns. It occurs when an infant inherits the gene for the sickle hemoglobin from both parents or the gene for sickle hemoglobin from one parent and the gene for another abnormal hemoglobin from the other parent. In SCD patients, the hemoglobin molecules in RBCs that carry oxygen throughout the body tend to cause the RBC walls to adhere to blood vessel walls. This leads to the painful sickle cell episodes that are the hallmark of SCD. Chronic end-organ damage occurs in the brain, lungs, kidneys, spleen, and liver and leads to premature death, with the median age at death for severely affected individuals occurring between 42 and 48 years.

NHLBI's current SCD portfolio includes research on the following topics: 1) development of methods for gene transfer and gene replacement in the hematopoietic stem cell; 2) characterization of interactions between sickle cells and the vascular endothelium; 3) improved understanding of hemoglobin gene switching, allowing increased production of HbF; 4) a phase III clinical trial of hydroxyurea in children with SCD to determine whether hydroxyurea can prevent the onset of chronic end-organ damage;

5) an epidemiologic study of the incidence of parvovirus B19 seroconversion in children with SCD; 6) an epidemiologic study of the adult patients who participated in the Multicenter Study of Hydroxyurea trial; 7) a study of non-myeloablative preparative regimens for bone marrow transplantation leading to mixed chimerism as curative therapy for severely affected SCD patients; and 8) a study of sibling cord blood banking and transplantation of cord blood-derived stem cells to cure severely affected SCD patients. In addition, the NHLBI intramural program continued a seroconversion study of B19 parvovirus in SCD patients to prepare for a recombinant vaccine trial of baculovirus-engineered empty capsids.

Progress in SCD research in FY 2001 was highlighted by a report of a gene therapy cure of the transgenic mouse model of SCD. Investigators at the Massachusetts Institute of Technology and the Albert Einstein College of Medicine announced the insertion of a β-HbA gene variant into hematopoietic stem cells in two transgenic SCD mouse models (Berkeley and SAD). The animals were able to produce the corrected hemoglobin cells for up to 10 months, with associated correction of hematologic parameters, splenomegaly, and prevention of urine concentrating defect. This experiment paves the way for additional animal studies and, ultimately, human clinical trials to find a safe way to neutralize the abnormal blood-producing gene before the introduction of gene-therapy-treated blood-producing cells.

Systemic Lupus Erythematosus (SLE)

SLE, or lupus, is an autoimmune disorder in which the body produces antibodies that harm its own cells and tissues. Typical SLE symptoms are fatigue, arthritis, fever, skin rashes, and kidney problems. Lupus affects more women than men. Patients with SLE have a higher incidence of thrombosis and spontaneous loss of pregnancy. Its cause and cure are unknown, but symptoms can be controlled with appropriate treatment, and most patients can lead an active life. As part of its broad program of research in hemostasis and thrombosis, NHLBI is supporting studies on the development of diagnostic tests in pregnant women with SLE. Recent studies suggest that circulating antibodies in lupus patients compete with a protein, annexin V, that forms an antithrombotic shield in the placenta. The result is that procoagulant phospholipids remain exposed, cause thrombosis in the vessels of the placenta, and lead to fetal loss.

Thrombotic Thrombocytopenic Purpura (TTP)

TTP is a potentially fatal disease characterized by low blood platelet levels and widespread platelet thrombi in arterioles and capillaries. Relapse is common in those who survive the acute phase. Both endothelial cell damage and intravascular platelet aggregation have been suggested in the pathogenesis of TTP. Microscopic examination of thrombi has revealed the presence of abundant von Willebrand factor (VWF), a plasma protein. An interaction between VWF and the platelet surface glycoprotein complex I is considered essential for the formation of a thrombus. VWF is synthesized as large polymers and then cleaved into smaller units by a plasma protease. NHLBI grantees have confirmed the presence of inhibitory antibodies to this enzyme in the plasma of some patients with TTP. Inhibition of the enzyme results in large multimers of VWF in plasma that can spontaneously aggregate platelets. NHLBI grantees succeeded in isolating a new metalloprotease, the ADAMTS 13 degradative enzyme inhibitor, and established that mutations in the gene that expresses ADAMTS 13 are the genetic basis of familial TTP. Efforts are being made to produce recombinant ADAMTS 13.

Research Initiatives

Ongoing Initiatives

- Adult Hydroxyurea Patient Followup Study (aka: Multicenter Study of Hydroxyurea in Sickle Cell Anemia [MSH] Patients' Followup)
- Beryllium-Induced Diseases
- Cellular and Molecular Mechanisms of Primary Pulmonary Hypertension

- Chronic Fatigue Syndrome: Pathophysiology and Treatment
- Clinical Research on Cooley's Anemia
- Comprehensive Sickle Cell Centers
- Creutzfeldt-Jakob Disease Assay Methods Development
- Developmental Processes in Differential Expression of Globin Genes
- Environment/Infection/Gene Interactions in Autoimmune Disease
- Genomic Applications for Heart, Lung, and Blood Research
- Homocyst(e)inemia and Atherosclerosis
- Human Umbilical Cord Blood Stem and Progenitor Cells: Collection, Storage, and Transplantation
- Immunogenetics of Inhibitor Formation in Hemophilia
- Mitochondrial DNA Mutations in Heart, Lung, and Blood Diseases
- Molecular Biology and Genetics of Sleep and Sleep Disor100ders
- Programs of Excellence in Gene Therapy
- Retinoid Treatment in Emphysema: Feasibility Studies
- SCOR in Hematopoietic Stem Cell Biology
- SCOR in Neurobiology of Sleep and Sleep Apnea, Airway Biology and Pathogenesis of Cystic Fibrosis, and Acute Lung Injury SCOR in Pathobiology of Fibrotic Lung Disease, Pathobiology of Lung Development, and Cellular and Molecular Mechanisms of Asthma
- SCOR in Pediatric Cardiovascular Disease
- Stem Cell Transplantation To Establish Allochimerism
- Strategies To Augment Alveolization
- Thalassemia (Cooley's Anemia) Clinical Research Network
- Thrombocytopenia: Pathogenesis and Treatment
- Tuberculosis Academic Award
- Vascular and Hematopoietic Development and Disease

Initiatives Started in FY 2001

Blood and Marrow Transplant Clinical Research Network

A new request for applications (RFA), initiated by NHLBI and cosponsored by NCI, organizes a network to accelerate research on the management of hematopoietic stem cell transplantation, standardize existing treatments, and evaluate new ones. A network of 14 interactive clinical centers and a data coordinating center provides a coordinated, flexible mechanism over a maximum period of 10 years to accept ideas and build consensus from the transplant community; develop protocols; expeditiously perform multicenter phase II and III clinical trials; provide information to physicians, scientists, and the public; and, in turn, improve stem cell transplantation therapy for diseases such as leukemia, SCD, thalassemia, and FA.

Genetic Aspects of Tuberculosis in Lung

A new RFA, initiated by NHLBI and cosponsored by NIAID, stimulates research on the genetic aspects of tuberculosis in the lung, exploiting advances in molecular biology and genomics research. Special attention is paid to the interaction between host and microbial genes and to the identification of genes or families of genes that determine virulence or latency or that determine reactivation of disease or resistance to antituberculous drugs. Of particular interest are studies with new biotechnologies such as microarrays, molecular beacon technology, and differential signature-tagged mutagenesis (DSTM) and studies involving innovative collaborations with computational biologists to identify genes that mediate the pathogenesis of tuberculosis and elucidate the responsible mechanisms. To encourage junior-level quantitative biologists to work on the genetic aspects of tuberculosis, the Mentored Quantitative Re

search Career Development Award (K25) has been included as a support mechanism.

Genetic Modifiers of Single Gene Defect Diseases

A new RFA, initiated by NHLBI and cosponsored by NIDDK, encourages studies to identify and characterize the genes responsible for modifying the clinical progression and outcomes of heart, lung, and blood diseases resulting from single gene defects. Examples of such single gene defect diseases are CF, SCD, hemophilia, α_1-antitrypsin deficiency, glucocorticoid remediable aldosteronism, Liddle syndrome, and cardiac myopathies, dysplasias, and arrhythmias that result in sudden cardiac death. The modifier genes are likely to encode a wide variety of proteins that interact directly with the disease gene, influence pathways involving the disease gene, or affect metabolic processes altered as a result of the disease gene defect. Identification of the genes responsible for these differences should lead to a better understanding of disease pathogenesis, earlier diagnosis, and improved treatment.

Novel Approaches to Enhance Animal Stem Cell Research

A new PA, cosponsored by 10 Institutes, encourages studies to isolate, characterize, and identify totipotent and multipotent stem cells from nonhuman biomedical research animal models and generate reagents and techniques to characterize and separate them from other cell types. The PA stresses innovative approaches to the problems of making multipotent stem cells available from various nonhuman sources, as well as innovative approaches to creating reagents that will identify them across species and allow for separation of multipotent stem cells from differentiated cell types.

Pathogenesis and Treatment of Lymphedema

A new PA, initiated by NHLBI and cosponsored by NICHD, NIAMS, and NCI, encourages investigation into the pathogenesis of and new treatments for primary and secondary lymphedema. The PA seeks to stimulate research on the biology of the lymphatic system; to characterize the pathophysiological mechanisms that cause the disease at the molecular, cellular, tissue, organ, and intact organism levels; and to discover new therapeutic interventions. This knowledge will improve early diagnosis of affected individuals, choice and timing of treatment, and genetic counseling.

Pediatric Heart Disease Clinical Research Network

A new RFA establishes a network of interactive pediatric clinical research centers to promote efficient evaluation of new treatment methods and management strategies that offer potential benefit for children with structural congenital heart disease, inflammatory heart disease, heart muscle disease, and arrhythmias. Therapeutic trials and studies involve investigational drugs, drugs already approved but not currently used, and devices, interventional procedures, and surgical techniques. The network approach, consisting of five or six clinical centers and a data coordinating center, is an effective, flexible way to study adequate numbers of patients with uncommon diseases such as congenital cardiovascular malformations. Efficiencies are achieved through standardizing procedures to recruit, characterize, monitor, and follow up on patients. Approximately 2,000 patients are expected to participate in 6–12 protocols over the 5-year project period. The network also serves as a platform to train junior investigators in pediatric clinical research and vehicle for rapid and widespread dissemination of findings.

Planned Initiatives

Animal Models of Antigen-Specific Tolerance for Heart and Lung Transplantation

A new PA in FY 2002 encourages the development of large-animal models of antigen-specific tolerance induction for heart and lung transplantation and small-animal models of tolerance induction for lung transplantation. Development of stable immune tolerance between donor and recipient would decrease morbidity and mortality caused by chronic rejection, toxic effects of immunosuppressive therapy, and GVHD.

Chemical Screens for New Inducers of HbF for SCD and Cooley's Anemia Treatment

A new PA in FY 2002 supports high-throughput chemical activity screens for new pharmacological inducers of HbF, with the long-term objective of developing better drugs to treat SCD and Cooley's anemia. Screens should include but not be limited to compounds in the short-chain fatty acid and carbonic acid classes. Promising compounds identified through these Small Business Innovation Research grants will later be subjected to toxicological and pharmacokinetic testing in primates.

Heritable Disorders of Connective Tissue

A new RFA, initiated by NIAMS and cosponsored by NHLBI in FY 2002, promotes research on heritable disorders of connective tissue caused by abnormalities in the molecules involved in the biosynthesis, processing, and degradation of structural macromolecules, as well as abnormalities in regulatory and signaling molecules that reside within the extracellular matrix. This initiative should increase understanding of and lead to novel therapeutic strategies for Marfan and Ehler-Danlos syndromes—diseases that involve alterations of the integrity of the connective tissue compartments within the wall of the blood vessel and the subsequent formation of aneurysms in the aorta and smaller arteries.

Multicenter Study of Hydroxyurea in SCD: Patient Followup Extension I

A renewal of an RFP in FY 2002 continues the followup study of the 299 adult patients who participated in the Multicenter Study of Hydroxyurea in SCD (MSH trial) from 1992 to 1995 to ascertain the long-term toxic effects of hydroxyurea usage in this population. The 240 patients known to be alive will be followed annually for 5 additional years at the 21 MSH clinical centers to determine health status, quality of life, incidence of malignancies, and birth defects in their offspring. Mortality rates will be compared with the mortality data from the Cooperative Study of Sickle Cell Disease adult cohort and the unaffected African American population. In addition, long-term efficacy of hydroxyurea will be estimated in terms of its effects on HbF, blood cell counts, and select organ function.

Plasticity of Human Stem Cells in the Nervous System

A new PA, cosponsored by NHLBI and three other Institutes in FY 2002, encourages studies on the plasticity and behavior of human stem cells and the regulation of their replication, differentiation, and function in the nervous system. Because of their ability to generate neurons and glia, stem cells are promising candidates for development of cellular and genetic therapies for neurological disorders, including neuroregulatory problems in heart, lung, and blood diseases, and sleep disorders. Studies will be encouraged to confirm, extend, and compare the behavior of human stem cells derived from different sources and ages or exposed to different regimens in vitro and in vivo. In addition, studies will be encouraged to develop methods for identifying, isolating, and characterizing specific human precursor populations at intermediate stages of differentiation into neurons and glia.

Stem Cell Plasticity in Hematopoietic and Nonhematopoietic Tissue

A new RFA, initiated by NHLBI and cosponsored by NIDDK and NINDS in FY 2002, encourages studies to elucidate and characterize the molecular and cellular mechanisms that influence stem cell plasticity or versatility. Stem cells are the most primitive bone marrow cells from which all blood cell types are derived. Studies are needed to identify genes responsible for maintenance of "stemness" and those responsible for initiating and/or maintaining the development of specific cell types. Human adult stem cells could potentially be exploited to become more embryoniclike and therefore useful for drug screening, replacement of diseased or injured tissue, and gene therapy.

Transactivation of HbF Genes for SCD and Cooley's Anemia Treatment

A new RFA, initiated by NHLBI and cosponsored by NIDDK in FY 2002, encourages studies to identify the transcriptional regulatory proteins involved in HbF gene activation, deter

mine their mechanisms of action and the induction mechanisms of the structural genes encoding the regulators, and identify drugs that induce HbF via action on the regulators. Better understanding of the molecular basis of HbF gene regulation and fetal-to-adult hemoglobin isoform switching in development will facilitate development of new approaches to cure β-chain hemoglobinopathies such as SCD and Cooley's anemia.

Transfusion Medicine/Hemostasis Clinical Research Network

In FY 2002, a new RFA establishes a network of interactive clinical research groups to promote the efficient comparison of new management strategies of potential benefit for children and adults with hemostatic disorders and to evaluate new and existing blood products and cytokines for treatment of hematologic disorders. Hemostasis, the arrest of bleeding from an injured blood vessel, requires the combined activity of vascular, platelet, and plasma factors counterbalanced by regulatory mechanisms to limit the accumulation of platelets and fibrin in the injured area. Hemostatic abnormalities may be congenital, immune mediated (e.g., ITP and TTP), or caused by coagulopathies resulting from chemotherapy, surgery, or trauma and can lead to excessive bleeding or thrombosis. The network will consist of a data coordinating center and up to 16 core clinical centers to perform multiple clinical trials.

Cell-Based Therapies for Heart, Lung, Blood, and Sleep Disorders and Diseases

A new RFA in FY 2003 will encourage basic research on stem cell biology and the use of stem cells in cellular therapies for the treatment of cardiovascular, lung, blood, and sleep disorders and diseases. Because of their plasticity, adult, embryonic, and fetal stem cells hold great potential for use in new strategies to regenerate and repair damaged or diseased cardiovascular, lung, and blood tissues and for sleep disorders. Areas supported will include basic biology and characterization of embryonic, fetal, and adult stem cells and progenitor cells important for heart, lung, blood and sleep disorders; use and differentiation of stem and progenitor cells for cell transplantation; stem cell homing to sites of tissue injury or specific tissue or organ sites, including mechanisms underlying the homing process; and tissue engineering with stem or progenitor cells.

Comprehensive Sickle Cell Centers

A FY 2003 renewal of an RFA will continue the operation of a nationwide network of collaborative, comprehensive centers in basic and translational research focused on developing cures or significantly improving treatments for SCD. The network of 10 centers and a statistics and data management core performs basic research, intercenter collaborative clinical research, and local clinical research focused on the most promising biomedical and behavioral therapeutic modalities. The centers also support career development of young investigators in SCD research and support services, including patient education, patient counseling, community outreach, and hemoglobin diagnosis. This is the eighth recompetition of a program established by a Presidential initiative and congressional mandate in 1972.

Hutchinson-Gilford Progeria Syndrome (HGPS): Exploratory/Developmental (R21) Grants

A new PA with review in FY 2003, to be cosponsored by four Institutes, will encourage studies to elucidate the molecular and mechanistic bases of HGPS, an incurable and terminal premature aging disorder characterized by short stature, abnormal skeletal and tooth development, sclerodermalike skin changes, and cardiovascular disease. Affected children usually die of heart attacks or strokes at an average age of 13. Little research has been conducted on the syndrome because it is extremely rare (about 1 in 10 million births), and access to the patient population has been limited. Fibroblast and lymphoblastoid cell lines from HGPS patients from 10 families will be available to awardees. Better understanding of the mode of inheritance, molecular basis, and pathomechanisms of HGPS could lead to new insights into mechanisms of development, aging, and vascular occlusive diseases.

Mechanisms of HbF Gene Silencing for Treatment of SCD and Cooley's Anemia

A new RFA will encourage studies in FY 2003 to delineate the mechanisms involved in HbF (γ-globin) gene silencing during normal human development and develop therapeutic approaches to inhibit silencing. Both *cis*- and *trans*-acting elements important in γ-globin gene silencing will be identified, and their mechanisms of action will be determined. Pharmacological or gene-based approaches to interfere with silencing may ultimately be pursued. Increased understanding of the molecular basis of HbF silencing will facilitate development of new gene-based therapeutic approaches to increase HbF in RBCs and thereby cure β-chain hemoglobinopathies.

Mesenchymal Stem Cell Biology

A new RFA will encourage studies in FY 2003 to conduct basic research on mesenchymal cell biology to find the basis for clinical application of mesenchymal stem cells (MSCs) to hematopoietic and nonhematopoietic stem cell transplantation. MSCs are pluripotent progenitor cells located in bone marrow that can differentiate into various nonhematopoietic tissues including bone, cartilage, tendon, fat, muscle, and early progenitors of neural cells. Preclinical studies suggest that MSCs facilitate hematopoietic stem cell transplantation while decreasing immune rejection of allogeneic transplants. To realize the therapeutic potential of these results, the initiative will support the identification of population and assay methods to characterize the clinical potential of candidate human MSCs and development of isolation and characterization standards for comparisons.

Molecular Target and Drug Discovery for IPF

A new RFA will encourage studies in FY 2003 to develop new therapeutic approaches for IPF. One approach to inhibit progression or reverse fibrosis in IPF patients is to identify new agents, ranging from small molecules to vaccines, that interact with previously identified molecules or pathways known to be involved in the development of fibrosis. Other promising approaches supported by this initiative are new technologies to identify additional molecular targets for treatment and identification of agonists or antagonists that interact with the previously or newly identified targets to attenuate, halt, or reverse the fibrotic process.

SCOR in Neurobiology of Sleep and Sleep Apnea and in Airway Biology and Pathogenesis of CF

A renewal of an RFA to foster multidisciplinary basic and clinical research in FY 2003 will enable basic science findings to be more rapidly applied to clinical problems of sleep and CF. The objective of the sleep SCOR is to integrate clinical research on the etiology and pathogenesis of sleep disorders, particularly sleep apnea, with molecular, cellular, and genetic approaches to the study of sleep. The objective of the CF SCOR is to use current knowledge of CFTR to promote advances in research on the pathogenesis of CF, the role of CFTR in airway biology, and development of new treatment strategies. Each SCOR must consist of three or more projects, all of which are directly related to the SCOR program topic. This will be the second and final 5-year solicitation for these two SCORs.

Program Activities

A task force meeting on research in pediatric cardiovascular disease was held in January 2001. The task force identified the following eight research priorities for the next 5 years:

- Fundamental studies of the formation of heart and blood vessels.
- Development and use of new and improved technologies to image the heart.
- Advanced repair of congenital heart defects in infants and children.
- Refined surgical treatment of human fetuses with heart defects.
- Exploration of stem cell biology for repair of heart tissues.
- Creation of improved biomaterials through tissue engineering.
- Translational research to enhance clinical care.

- Definition of the childhood antecedents and risk factors for atherosclerotic heart disease in adults.

The Lamposium 2001 symposium, held in March 2001 in Cincinnati, Ohio, was cosponsored by NHLBI and the LAM Foundation.

An RFA meeting for Clinical Research for Cooley's Anemia and Biology of Iron Overload was held in April 2001.

The workshop AAT Deficiency: The Challenge of Genetic Conditions, held in June 2001 and cosponsored by the Alpha One Foundation, NHLBI, NIDDK, and ORD, was designed to promote multidisciplinary understanding of psychosocial and scientific challenges of AAT deficiency.

The workshop Host Response in Sickle Cell Disease, held in June 2001, discussed the clinical manifestations of problems with the immune response in SCD. Specific topics included resistance to *Pneumococcus,* genetic modifiers of the immune response, loss of splenic function in SCD, and response to encapsulated bacteria, iron overload, autoimmune disorders, developmental immunity, white blood cell function in SCD, and consequences of chronic transfusions. It was recommended that research be pursued to ascertain the genetic factors that modify phenotypic differences in the responsiveness of SCD patients to infections.

The workshop Protein Processing and Degradation in Pulmonary Health and Disease, held in September 2001 and cosponsored by NHLBI and ORD, was designed to evaluate the current state of knowledge of protein biosynthetic processing and intracellular degradation.

A working group meeting on targeting technologies for repair of single nucleotide mutations in single gene-defect blood diseases, held in September 2001, assessed the potential of various approaches for correction of pathogenic single-nucleotide mutations in SCD, β-thalassemia, hemophilia A and B, and hemochromatosis.

A research training program designed for clinicians interested in performing biomedical research related to PPH will be cosponsored by NHLBI and the Pulmonary Hypertension Association. The training will be supported by the Mentored Clinical Scientist Development award (NIH activity code K08). A working group on translational research in PPH, sponsored by ORD and NHLBI, is planned for FY 2003.

Problem Areas

α_1-Antitrypsin Deficiency

Research must include better animal models of the disease, identification of biomarkers, and development of chemical chaperones that could specifically enhance the secretion of the mutant α_1-antitrypsin protein.

ARVD

A concerted multilaboratory program that combines basic, clinical, and genetic approaches is needed to identify the causes of this highly lethal form of cardiomyopathy. Once contributing factors are found, the challenge will be to search for therapies. Additional clinical centers, and perhaps a national registry, would be useful to investigators who are already studying the origins of and potential treatments for ARVD.

CCHS

A significant limitation is the difficulty in recruiting CCHS subjects for clinical research. CCHS is a very rare condition, often presenting within a few hours of birth. Only an estimated 150 CCHS patients exist worldwide. Relocation to clinical research sites is difficult because of the spectrum of clinical symptoms associated with CCHS and related dysfunction of the autonomic nervous system.

CDH

As a result of advances in ultrasonography, CDH is now diagnosed before birth with increasing frequency. Development of microsurgical techniques has enabled surgical repair in utero. With multiple options available to families, accurate counseling on the expected outcome is crucial. Scientific information must be

provided to assist affected families in making decisions about management.

Congenital Heart Disease

Long-term followup studies are required to answer certain questions, but because congenital heart disease is often repaired in infancy, such studies are difficult to initiate. Additional research is needed on adult congenital heart disease, pulmonary malformations in congenital heart disease, and pediatric ventricular assist devices.

CJD

A standardized reference material repository is needed to validate assay systems to detect TSE. Materials under consideration to calibrate in-house reference materials of individual laboratories to a single international standard include both human and animal brain tissue and blood. Blind panels are needed for validation of all assays, specifically their sensitivity, reproducibility, and predictive abilities. NHLBI is developing an initiative to support establishment of a standardized reference TSE material repository.

GVHD

Promising agents for treating GVHD are also under investigation for use in other diseases, for example, arthritis. Pharmaceutical companies are reluctant to give transplant research investigators access to these investigational drugs for fear that complications experienced by HCT patients will interfere with the approval process for new agents.

Infectious Myocarditis

Dilated cardiomyopathy is thought to be a consequence of myocarditis in a subgroup of genetically predisposed people. Identification of the genetic basis for more severe disease may enable clinicians to target patients who would benefit from more aggressive therapy. More specific and sensitive noninvasive methods for diagnosis are needed. The current gold standard is endomyocardial biopsy, but this procedure suffers from limited specificity and sensitivity. Also, the concept of myocarditis as an autoimmune phenomenon is supported by studies linking persistence of viral RNA in the myocardium to the induction of autoantibodies. More research is needed to determine the effectiveness of immunosuppressive modalities in myocarditis. NHLBI-supported investigators are tackling several of these problem areas.

LQTS

Access to and identification of sufficient numbers of new patients for studies remain a problem. Identification of mutant-gene carriers would be greatly facilitated by accurate means of screening individuals in afflicted families for specific founder mutations. Improved means of identifying new mutations in the various genes involved would also be helpful. Investigators are working to increase the visibility of an international LQTS registry in minority communities.

LAM

LAM tissue is scarce, and cell lines are difficult to establish and maintain. Currently, no animal models of LAM exist.

PPH

Detailed understanding of BMPR-2 gene function has not yet been achieved, and how this gene may cause the structural and functional changes in the lungs of PPH patients is not clear. Although progress is being made, no animal models have been developed that completely mimic PPH in humans. The etiology and pathogenesis of PPH must be understood before successful therapies can be developed. Current therapies are cumbersome and expensive and are not effective for all patients. Innovative mechanisms are needed to accelerate the translation of new findings into better PPH treatments.

SLE

The fear of miscarriage is a great concern for many pregnant women with SLE. Anticoagulation therapy with antibodies to phospholipids must be evaluated for high-risk pregnant women.

National Human Genome Research Institute (NHGRI)

Overview

NHGRI's mission is to understand the structure and function of the human genome and the role it plays in human health and disease. To that end, NHGRI supports several activities, including the Human Genome Project (HGP), an international research effort to sequence the human genome and determine the function of the genes contained within it. Publication of the initial sequence and analysis of the human genome in February 2001 was a historic scientific achievement. The sequence information from the HGP has been continuously, immediately, and freely released to the world, with no restrictions on its use or redistribution. This information is a major resource for all areas of basic and applied biomedical and behavioral research in the 21st century. The HGP is already producing research tools and information that are leading to improved detection and diagnosis of genetic disorders.

Using the information and tools produced by the HGP and other approaches, scientists in the Institute's intramural research program are developing techniques to study the fundamental mechanisms of genetic disorders and genetic factors involved in common and rare diseases. These cutting-edge approaches are yielding new knowledge about many rare diseases and their prevention, diagnosis, and treatment.

Scientific Advances in Tools for Gene Discovery

Biochip technologies

One of the many outcomes of the HGP has been the need to develop high-throughput technologies for analyzing many genes and proteins at once. High-throughput biology research has become routine with the development of cDNA micro-array technologies that enable analyses of expression of thousands of genes at once. As useful as this is, the cDNA microarray data often only provide hypotheses to be tested in future studies or candidate target genes whose involvement in a biological or clinical process should be further investigated. Often the number of genes to be tested ranges from hundreds to thousands. The translational genomics section of the HGP is developing novel tools, technologies, and bioinformatic solutions for validating functional genomics data of cancer. The following approaches are used:

1. Results from different kinds of biochips are integrated to define the most important gene targets first. For example, researchers are using data from gene copy number (by CGH [comparative genomic hybridization] microarrays) to prioritize genes that may be targets of a genetic rearrangement in cancer.
2. New high-throughput functional validation is being developed, such as the use of cell arrays to test the function of hundreds of genes at once on the cell phenotype.
3. Pharmacogenomic profiling of cancer cell lines is performed to identify genes, pathways, and molecular mechanisms that are important for therapy response.
4. Researchers are developing bioinformatic tools to visualize gene expression data and integrate these data with results from other biochip technologies.

Genetics of Human Diseases

Scientists in NHGRI's Division of Intramural Research apply genomic tools to the study of human genetic diseases, many of which are rare. Research progressed in the following areas.

Inherited Disorders of the Immune System

The NHGRI Genetics and Molecular Biology branch is conducting a research program to find the causes of and develop better treatments for inherited disorders of the immune system. These include immunodeficiencies, in which gene defects impair the ability of the immune system to

fight infections, and disorders of immune cell regulation, in which autoimmunity may be seen. Current areas of investigation include severe combined immunodeficiency, mucocutaneous candidiasis, hyper-IgE syndrome, certain inherited autoimmune diseases, including variants of autoimmune lymphoproliferative syndrome, and genetic determinants of susceptibility to human immunodeficiency virus (HIV)/AIDS.

Severe Combined Immunodeficiency (SCID)

SCID is a rare but devastating complete lack of T-cell and B-cell immunity, also known as the Bubble Boy disease. NHGRI scientists discovered the gene for the most common form of SCID, the interleukin-2 receptor γ gene *(IL2RG),* which encodes the common γ-chain of receptors for several lymphocyte growth factors or cytokines. When this gene is defective, lymphocytes cannot develop normally, and affected infants therefore have frequent, severe infections that are ultimately fatal unless the immune system can be restored. Scientists are analyzing the expression and function of the common γ-chain protein. Carrier testing and genetic counseling can then be provided, as well as a prenatal diagnosis, which makes affected infants eligible for improved early treatments.

In addition, scientists have developed and tested methods for correcting the genetic defect in X-linked SCID by gene transfer. Bone marrow transplantation is often life saving, and gene transfer is a promising treatment. Clinical trials of human gene transfer are planned to treat patients with X-linked SCID who were not helped by bone marrow transplantation.

Hyper-IgE Syndrome (Job Syndrome)

Hyper-IgE syndrome is an enigmatic rare condition characterized by recurrent skin abscesses, recurrent pneumonia with development of lung cysts, and extreme elevations of serum IgE. The specific immune defect has not been discovered; however, NHGRI scientists have found that the syndrome can be inherited as an autosomal dominant disorder, and therefore genetic studies may help find the cause. NHGRI and NIAID scientists have arrived at a new clinical understanding of the condition as a multisystem disorder with immune, dental, and skeletal abnormalities. It has variable expressivity and penetrance. Genomewide linkage studies show at least three loci in the human genome that may be associated with hyper-IgE syndrome. Scientists have also mapped dominant mucocutaneous candidiasis to human chromosome 2p.

Autoimmune Lymphoproliferative Syndrome (ALPS)

ALPS is a newly discovered syndrome in which patients have large lymph nodes and spleens, autoimmune disease, increased numbers of a rare type of lymphocyte called $CD4^-/CD8^-$ T cells, and defects in programmed cell death of their lymphocytes. NIH research has shown that people with ALPS have a high risk of lymphoma. NHGRI and NIAID scientists have discovered that most patients with this condition have inherited defects in the apoptosis mediator *Fas.* The position of mutations within the *Fas* gene influences the severity of ALPS and whether family members with the same mutation are likely to have symptoms. Mouse models for ALPS combined with studies of family members can show how varying genetic background influences the disease manifestations.

Familial Mediterranean Fever (FMF)

FMF is an inherited disease associated with periodic fever and abdominal pain. The aim of this study is to use a transgenic mouse model to understand the role of the human FMF gene in the pathogenesis of the disease. The function of the protein made by this gene is still unclear, although it is likely to play an important role in inflammatory and immune response. FMF occurs frequently in the Middle East and, if untreated, can be fatal due to amyloidosis and renal failure. NHGRI scientists have cloned this gene and found that patients with FMF have point mutations in this gene. The gene is expressed specifically in maturing granulocytes, one of the target tissues of the FMF disease process.

MEFV encodes for a protein of unknown function. Scientists have generated transgenic mice with a deletion of the *MEFV* gene to understand the gene's normal function. Mice with homozygous *MEFV* deletions grew normally and have

not shown any visible defects or suffered any specific diseases. However, detailed analysis of granulocytes indicated that the control of apoptosis, a process through which unwanted cells can be recycled, is defective in these animals. Scientists are also generating transgenic mice with point mutations in the *MEFV* gene, mimicking those found in FMF patients. Such mice can be used to confirm the importance of the mutations for induction of the disease and to study the pathophysiology of the disease, which may lead to better treatment of FMF patients.

Developmental Disorders

Polydactyly Syndromes

A group of syndromes that include polydactyly with other malformations is the subject of a clinical molecular study. These disorders include Pallister-Hall syndrome, Greig cephalopolysyndactyly syndrome (GCPS), McKusick-Kaufman syndrome, and Bardet-Biedl syndrome. Manifestations of these disorders include polydactyly, central nervous system malformations (with or without mental retardation and seizures), craniofacial malformations, and visceral malformations such as renal malformations and congenital heart defects. Researchers are studying these disorders using a translational approach that begins with careful clinical evaluation of the phenotypes by physical examination, imaging studies that include radiography, ultrasound, magnetic resonance imaging, and computed tomography. This work has demonstrated the cause of Pallister-Hall syndrome and that Bardet-Biedl and McKusick-Kaufman syndromes can both be caused by mutations in the same gene. In addition, researchers have shown that in GCPS, patients with large deletions are more likely to have developmental delay or delayed speech.

Molecular Genetics of Anabaptist Diseases

The Old Order Amish and Mennonites represent a cultural and genetic isolate and are subject to a number of extremely rare, and perhaps unique, genetic diseases. The disease under study for the past year is Amish microcephaly, a disease that is affecting fewer than 75 individuals, causing severe prenatal brain maldevelopment and hypoplasia. The disorder is lethal, usually within 6 months. Progress has been made in the genetic and physical mapping of this disease, and collaborative efforts are under way to characterize the central nervous system pathology.

Proteus Syndrome

Proteus syndrome is a rare disorder of segmental overgrowth, affecting fewer than 100 patients worldwide. It is sporadic and associated with severe complications, including sudden death due to deep venous thrombosis and pulmonary embolism, progressive orthopedic complications, and tumor predisposition. A natural history study is under way to characterize the full range of the disorder's manifestations and to test clinical interventions that can ameliorate the symptoms and reduce the mortality of this disorder.

Lenz Microphthalmia Syndrome

Researchers at NHGRI are investigating the clinical and molecular basis of Lenz microphthalmia syndrome, a rare disorder causing small or absent eyes, mental retardation, and other anomalies. Researchers have identified a large family affected by this disorder and have mapped the gene to the short arm of the X chromosome. The results show that Lenz microphthalmia is probably an amalgam of two disorders, because another family with this disorder was mapped to the long arm of the X chromosome. Researchers are using positional cloning to isolate the gene that is altered in the condition; so far, 20 candidate genes have been identified and are being sequenced. The results of this research should enable development of accurate diagnostic tests for Lenz microphthalmia syndrome.

Lowe Syndrome

Lowe syndrome is a rare X-linked disorder characterized by congenital cataracts, developmental delay, and Fanconi syndrome of the renal tubules. The defect is a deficiency in an enzyme, a phosphatidylinositol-4,5-bisphosphate 5-phosphatase localized in the Golgi complex, particularly the *trans*-Golgi network. NHGRI scientists are investigating the relationship between this enzyme deficiency and the clinical

phenotype via cellular and animal models. NHGRI is also hosting a major clinical conference this year in cooperation with ORD, the Lowe Syndrome Association (U.S.A), and the Lowe Syndrome Trust (U.K.) to address problems in management and treatment of complications of Lowe syndrome.

Alagille Syndrome (AGS)

Scientists at NHGRI have shown that mutations in the *Jagged1* gene *(JAG1)* are responsible for AGS, a developmental disorder affecting multiple organ systems including liver, heart, eye, face, and vertebrae. To understand the role of *Jaggeds* in vertebrate development and to understand how alterations in their function lead to AGS in humans, scientists have isolated and characterized *Jagged* genes from zebrafish. There are three *Jaggeds* in zebrafish, and they exhibit distinct expression patterns during development. Researchers are blocking expression of Jaggeds with antisense oligonucleotides to evaluate the function(s) of the Jagged proteins in vertebrate development.

Smith-Magenis Syndrome (SMS)

SMS, a rare microdeletion syndrome characterized by deletion on the short arm of chromosome 17, is associated with a distinct phenotype of physical features, developmental delay, speech delay with or without associated hearing loss, clinical signs of peripheral neuropathy, and neurobehavioral problems, including sleep disturbance, outbursts, and self-injurious behaviors. More than 200 individuals worldwide representing diverse ethnic backgrounds have been identified with the syndrome. Using existing physical maps and comprehensive clinical analysis of the physical, cognitive, and neurobehavioral aspects of SMS, the SMS research team seeks to define the natural history and pathophysiology of SMS across the lifespan and identify genes in the chromosome 17p11.2 region that contribute to physiological and functional aspects of human cognition, speech/language development, and behavior. The SMS team is funded by a Bench to Bedside Award given by the NIH Clinical Center in 1999.

Left-Right (L-R) Axis Malformations

A study of the complex genetics of L-R axis malformations is investigating genes associated with common phenotypes of L-R disorders, including situs inversus, heterotaxia, and organ isomerism. L-R defects can result from either environmental or genetic causes. Investigators at NHGRI aim to determine the genes responsible for both normal and abnormal L-R axis formation through the study of patients with these disorders. Mutations in genes such as *ZIC3, LEFTY A,* and activin type 2B receptor gene *(ACVR2B)* are responsible for several familial and sporadic cases of heterotaxia. Many additional genes important for L-R development will probably be identified in the search for genetic causes of laterality disorders. Recently, NHGRI scientists have identified the human *CFC1* gene as causing laterality defects and are studying this and other genes in individuals with cardiac anomalies.

Hirschsprung Disease

Animals heterozygous for mutations in the *SOX10* transcription factor exhibit multiple defects in neural crest development, including fewer melanocytes in the skin, absence of myenteric ganglion in the colon, and association with deafness. Hirschsprung disease, a human congenital disorder, also exhibits rectocolic aganglionosis and can be associated with hypopigmentation caused by *SOX10* mutations. Thus *SOX10* and other neural crest mutant mice serve as models for this disease. Involvement of *SOX10* in Hirschsprung disease and other neural crest-related disorders will be explored.

Camptodactyly–Arthropathy–Coxa Vara–Pericarditis (CACP) Syndrome

Scientists have identified mutations in a gene previously known as megakaryocyte growth and stimulating factor, which causes CACP syndrome. CACP is an autosomal recessive disease whose basic underlying defect is synovial hyperplasia, which leads to several clinical phenotypes, mainly loss of proper joint growth and function. Scientists have made a mouse knockout construct and are currently studying these animals to see whether they can replicate the

human phenotype in mice. This replication will aid understanding of joint development and enable scientists to identify the basic molecular components responsible for CACP.

Congenital Disorders of Glycosylation (CDG)

CDG is a group of metabolic disorders characterized by a wide range of phenotypic presentations, from severe developmental delay and systemic manifestations to only gastrointestinal symptoms and normal development. CDG results from defective N-linked oligosaccharide synthesis: a pathway with approximately 200 steps on which different types of CDG result from a disruption in any individual step. NHGRI scientists are identifying new patients with CDG and conducting studies to determine its pathogenic basis for novel cases and to define the relationship between genotype and phenotype in these patients. NHGRI scientists expect to elucidate the correlation between the phenotype, glycobiology, and genes involved.

Neurological Disorders

Batten Disease

Juvenile neuronal ceroid lipofuscinosis (NCL type III), known as Batten disease, is a degenerative neurological disease resulting from a lysosomal storage disorder. The Batten gene, *Cln3,* encodes a protein, the function of which is not really known.

Scientists at NHGRI have previously created a mouse model carrying a deletion of the *Cln3* gene, which encodes a transmembrane lysosomal protein of unknown function. The mouse has the same biochemical abnormalities seen in human patients with Batten disease. Work in yeast performed at the University of Rochester has revealed that the yeast ortholog of *Cln3* is a vascular protein that, when deficient, abnormally lowers the vascular pH. NHGRI scientists hypothesized that humans (and mice) deficient in *Cln3* might store lipofuschin in their lysosomes because of an abnormal depression of lysosomal pH that interferes with degradative enzyme function. Scientists have started a treatment protocol of mice with chloroquine, an alkaline base that accumulates in the lysosomes, to see whether the biochemical abnormalities seen in Batten disease can be corrected by this widely used and characterized drug.

Presenile Familial Dementia With Neuroserpin Inclusion Bodies (FDNIB)

This project involves the study of the novel familial neurodegenerative disorder FDNIB. The disorder, which has a characteristic clinical course of progressive dementia and neurologic involvement, was defined in one extended family. Neuroserpin is a strong candidate gene for this disorder, and a mutation is present in this large kindred. The project will characterize the clinical phenotype, delineate the natural history of the disorder, and explore genotype/phenotype correlation in the index family. Families with immunohistopathologically neuroserpin-positive neuronal inclusion on autopsy/biopsy in affected members or with familial presenile dementia with neurologic features consistent with the original FDNIB family will be enrolled.

Hyperparathyroidism-Jaw Tumor (HPT-JT) Syndrome

Using recombination mapping, a combined genetic analysis of HPT-JT by seven institutions, including the Karolinska Institute in Switzerland and the Leiden University Medical Center in the Netherlands, has refined the genetic locus to a region found on chromosome 1. With data from the publicly available draft human genome sequence, researchers at NHGRI have identified and are in the process of characterizing a total of 56 potential candidate genes, which map within the HPT-JT critical interval. These candidate genes are being characterized for tissue distribution and transcript length by Northern blot analysis. All partial transcripts are being extended into full-length cDNAs. Candidate genes were prioritized for mutation analysis based on expression pattern and available functional information. Mutation screening is being carried out on patient genomic DNA via direct sequencing of exons. Candidate genes are being screened in the hope of identifying disease-associated mutations in HPT-JT patient DNA samples. Identifying the HPT-JT gene could lead to early diagnosis and identification of novel therapies for this disease.

NHGRI researchers are also collaborating to narrow the region on chromosome 1 where the locus of HPT-JT has been mapped to eventually clone and characterize this gene. In FY 2001, analyses were performed on new genetic markers for pedigrees from several sites, and the critical region was further refined. A newly developed haplotype method for detecting association has been applied to these data, giving further evidence to narrow the critical region. Researcher collaborators at the University of Pisa, Italy, have performed statistical genetic studies to help identify this gene. The gene is believed to be located in the region predicted by statistical analyses to be the most likely location. Linkage and cloning results are being prepared for publication. NHGRI researchers plan to use these data to examine questions about our haplotype-sharing method and its uses in localizing disease genes.

Vision Disorders

Cataract and Craniofacial Anomalies Syndrome

A new rare syndrome involving congenital cataracts and craniofacial anomalies in an inbred Saudi Arabian family has been identified. The most prominent feature is failed closure of the fontanels and sutures, and, at birth, the anterior fontanel is large due to open sagittal and metopic sutures. The second major feature is posterior Y-shaped structural cataracts that are congenital or develop over time. Chromosomal and biochemical studies were normal. A genomewide screen was performed using 387 markers on 21 DNA samples at the Center for Inherited Disease Research. Efforts to fine map the gene were completed with identification of a single candidate gene. A search for candidate loci is under way, and tests for association will attempt to narrow the region and to subsequently clone the gene.

Rieger Syndrome

A continuing area of interest for NHGRI researchers involves the homeodomain family of proteins, which play a fundamental role in a diverse set of functions that include body plan specification, pattern formation, and cell fate determination during metazoan development. Members of this family are characterized by a helix-turn-helix DNA-binding motif known as the homeodomain. Homeodomain proteins regulate various cellular processes by specifically binding to the transcriptional control region of a target gene. These proteins have been conserved across a diverse range of species, from yeast to human. Several inherited human disorders are caused by mutations in homeodomain-containing proteins. One specific homeodomain protein, FOXC1, is implicated in Axenfeld-Rieger malformations. Patients with Axenfeld-Rieger malformations typically show a spectrum of ocular findings, including iris hypoplasia, a prominent Schwalbe line, iris adhesions, and goniodysgenesis. The most severe cases show elevated intraocular pressure, leading to the development of glaucoma. Work is continuing in this area so that these eye-related mutations and their net effect on vision can be better understood.

Behavior Disorders

Attention Deficit Hyperactivity Disorder (ADHD)

ADHD is a disorder of childhood and adolescence, sometimes with serious sequelae in the adult years. An international collaboration with scientists at the University of Antioquia, Colombia, was established in FY 2000 to study the genetics of ADHD in a Hispanic-Amerindian population isolate. To study the hypothesis that ADHD is a genetically influenced brain disorder, a genomewide search for loci linked to ADHD is being undertaken. To this end, 100 densely affected multigenerational Hispanic families are being recruited to the study. Participants undergo a battery of psychological tests and have blood drawn for the linkage analysis and positional cloning studies that will be used to search for genes associated with ADHD. In FY 2001, researchers completed information on five large, multigeneration Hispanic families. Simulation studies on 27 informative pedigrees indicate excellent power to detect linkage. The Center for Inherited Disease Research will analyze DNA from members of these families, and the study will continue in FYs 2002 and 2003.

Endocrine Disorders

Multiple Endocrine Neoplasia Type 1 (MEN1)

MEN1 is a cancer syndrome characterized by multiple tumors of the parathyroid, pancreatic endocrine, and anterior pituitary tissues. Previous research shows that inherited mutations in the *MEN1* gene are responsible for this syndrome. Researchers have also shown that the MEN1 encoded protein, menin, resides in the nucleus, binds the transcription factors *JunD* and NF-κB, and can repress JunD and NF-κB-induced transcription. *MEN1* orthologs from several species have been identified, including from mouse and *Drosophila.* Researchers have developed a mouse knockout model that has a tumor phenotype remarkably similar to the human MEN1 disease. In addition, tissue-specific transgenic expression and knockout models are being developed in *Drosophila,* which are expected to help understand the functional role of menin. Efforts to study changes in global gene expression have led to identification of nearly 50 transcripts in cells lacking menin. The importance of these transcripts in mapping out the pathway of menin biology can now be explored.

Meetings and Workshops

CDG Type 1A

CDG is a group of rare metabolic disorders with a multsystemic clinical presentation and is found in about 300 cases worldwide. The metabolic basis of CDG type 1A, the most common type, is a deficiency of phosphomannomutase with mutations defined in its gene, *PMM2.* This reflects defective synthesis of N-linked oligosaccharides with clinical manifestations—a direct result of the role of N-linked glycans in human embryogenesis and physiology. Therapeutic trials with mannose have shown some changes in laboratory tests, but no major clinical changes were seen in these children. However, at a recent CDG meeting, a report was presented in which two children with CDG had been treated with mannose and were making significant clinical improvement. This report renewed interest among CDG clinical experts of the need for a randomized therapeutic trial for children with CDG. Alternative therapeutic options explored as well.

Additional Activities

Genetic and Rare Diseases Information Center

To respond to the public's need for information on genetic and rare disorders, NHGRI and ORD launched the Genetic and Rare Diseases Information Center in early 2002. The Information Center focuses on meeting the information needs of the general public, including patients and their families, health-care professionals, and biomedical researchers. The purpose of the Information Center is to 1) serve as a central, national repository of information on genetic and rare diseases, conditions, and disorders; 2) collect, update, and disseminate information on the diagnosis, treatment, and prevention of genetic and rare disorders; and 3) coordinate with organizations and associations interested in genetic and rare disorders to explore networking capabilities, avoid duplication of effort, and identify information gaps.

National Institute of Mental Health (NIMH)

Overview

NIMH's mission is to reduce the burden of mental illness and behavioral disorders through research on mind, brain, and behavior. Serious mental disorders such as schizophrenia, depression and bipolar disorder, and anxiety disorders are common and the principal foci of the clinical and clinically inspired basic research that NIMH supports and conducts. However, the Institute's scientific programs also address several conditions that meet rare disease criteria, including the eating disorders anorexia nervosa and bulimia nervosa, suicide, prepubertal and adolescent bipolar disorder, pediatric human immunodeficiency virus (HIV)/AIDS, and body dysmorphic disorder.

Recent Scientific Advances

Anorexia Nervosa (AN) and Bulimia Nervosa (BN)

Eating disorders are often chronic, relapsing disorders that have some of the highest death rates of any psychiatric illness. Recent studies have shown that AN and BN run in families, and studies in twins suggest a genetic basis for the high rate of these eating disorders in certain families; in other words, 50–80% of the factors contributing to the development of eating disorders are genetic. This means that AN and BN may be as heritable as schizophrenia and bipolar disorder, which have long been regarded as exhibiting a strong degree of genetic vulnerability.

Twin, family, and genetic studies support the possibility that some underlying trait, such as a vulnerability to an imbalance in the serotonin system, may place a person at risk for developing an eating disorder. Studies have established that the leptin system also is fundamental in the regulation of energy balance and neuroendocrine function and that the leptin and serotonin systems may interact to influence ingestion. Research in an animal model of AN has implicated the ovarian hormone estradiol in reducing food intake, in addition to its more familiar role in regulating menstrual cycles. However, AN and BN may also have pathophysiological differences, in that recent cerebrospinal fluid studies have shown low levels of the pro-digestive hormone gastrin releasing peptide only in BN, whereas low levels of the appetite stimulant galanin seem to occur only in AN. Current studies are examining the role of various hormone levels in the modification of taste signals, which could increase understanding of the way ingestion-related information is processed in individuals with AN and BN.

Individuals with AN often exhibit symptoms of other psychiatric disorders, such as major depression and obsessive-compulsive disorder, which respond favorably to medication. It has been somewhat puzzling that medical treatment for AN has been consistently unsuccessful. One possible reason for the lack of medication effectiveness may be the associated neurochemical changes caused by starvation. Small treatment studies with the selective serotonin reuptake inhibitors fluoxetine and sertraline have recently demonstrated some efficacy and support the importance of the serotonergic system in the etiology of the disorder.

NIMH-supported investigators are conducting a clinical trial at four collaborating sites to evaluate differing sequences of treatments for BN. Some of the 324 bulimic women in this study are randomly assigned to first receive cognitive behavioral therapy and then added antidepressant medication (fluoxetine) if the psychotherapy alone does not produce remission of clinical symptoms (considered a state-of-the-art approach). Other patients are initially assigned to a supervised self-help program and receive medication only if they do not experience sufficient improvement. This stepped-care approach is considered readily implementable. The study aims to clarify whether either sequenced treatment model leads to superior clinical outcomes overall and whether either is preferable from a cost-effectiveness perspective.

Efforts to prevent eating disorders have been limited by difficulties in refining specific risk factors. Two separate randomized prevention trials have identified body-image concerns and unhealthy weight-control methods as symptoms to target in preventing the onset of more severe forms of eating disorders. Both of these trials are school based (high school and college), target young women, and use the Internet as a resource for monitoring attitude and knowledge change.

Suicide Deaths

Suicide is rare, accounting for 1.3% of total deaths in 1999. Nearly 90% of the time, fatal self-injuries occur in the context of a mental and/or substance abuse disorder. Statistics for 1999 indicate that 29,199 Americans took their own lives; however, for every suicide death, there are an estimated 8–100 suicide attempts. Individuals who complete suicide have overlapping but quite distinct characteristics compared with those who attempt suicide. For example, estimates of attempted suicide indicate that twice as many women attempt suicide as men; however, five times as many men as women actually die by suicide.

To best determine risk factors for suicide deaths compared to attempted suicide, researchers have focused on postmortem research methods to develop both psychological and biological risk profiles. NIMH has funded psychological autopsy research for youth and is currently funding a study focused on older adults. Youth suicide victims are more likely to have a mood, conduct, and/or substance abuse disorder and a history of past suicide attempts. In contrast, older suicide victims are more likely to have a later onset mood disorder or a physical illness but less likely to have a substance abuse disorder or to have made suicide attempts. NIMH-supported long-term studies of depression have found that recurrent hopelessness is a key risk factor for poor treatment adherence and consequent death by suicide.

NIMH-supported research on biological risk factors continues to provide compelling new evidence of clear differences in the brains of individuals who commit suicide. Previous neuroimaging findings in people with major depression revealed decreased volume and altered metabolism in the cerebral cortex. Studies in postmortem brains are finally able to provide detailed characterizations of this phenomenon at the cellular level. The most recent evidence shows that brain cell size and density are also decreased in specific regions of the cortex. To understand how such changes may occur, investigators have been studying measurable changes in brain chemistry, which are revealing clear differences in specific classes of neurotransmitter systems (e.g., serotonin, dopamine, and noradrenaline). These investigations have focused on how the regulatory mechanisms of these neurotransmitters and their receptor molecules differ in brains of suicide victims compared with normal individuals. Such refined investigations suggest a complex but interesting conclusion about the neurobiology of suicide and depression. Serotonin and dopamine levels clearly are significantly different in brains of suicide victims who were also suffering from major depression, which is consistent with earlier findings. However, the brains of a population of suicide victims who were not depressed also show differences in serotonin and dopamine measures, but the differences are not the same as for depressed suicide victims. This is a profound finding because it suggests a neurobiological risk factor for suicide that is independent of major depressive illness and other mood disorders. The most recent findings in animal studies suggest that the abnormal molecular regulation of serotonin receptor production seen in the brains of suicide victims may be reversible with antidepressant drugs such as Prozac.

Prepubertal and Adolescent Bipolar Disorder

In the past year, NIMH intramural investigators devised a phenotyping system for childhood bipolar disorder to address the contemporary confusion and controversy regarding making the diagnosis in children. A paper describing the new phenotyping approach is being prepared for publication. The investigators are analyzing data on approximately 100 children that appear to validate some aspects of the phenotyping system; namely, children in the narrow phenotype

(those having well-defined episodes of mania) are significantly more likely than those in the broad phenotype (who have a chronic irritable course) to have classic manic symptoms, history of psychosis, history of a suicide attempt, and a parent with bipolar disorder. These findings are also being prepared for publication.

The NIMH intramural investigators recently received approval to conduct a trial of clozapine in children and adolescents with treatment-resistant bipolar disorder. The same team is developing paradigms relevant to childhood bipolar disorder for use in functional magnetic resonance imaging.

Body Dysmorphic Disorder (BDD)

BDD, a preoccupation with an imagined or slight defect in appearance, is a chronic disabling and costly disorder that can lead to repeated hospitalizations and suicide. Although reliable data regarding prevalence are absent, BDD appears to be rare according to reports that people with BDD infrequently seek treatment from health professionals. However, some evidence suggests BDD may be more common than previously thought but that afflicted individuals are reluctant to discuss their symptomatology. NIMH is currently funding the first controlled treatment study for BDD, a 12-week double-blind study in which the efficacy of fluoxetine and placebo are being compared. Preliminary results indicate that 54% of participants randomized to fluoxetine were significantly improved after treatment versus 26% of participants randomized to placebo. No serious adverse events including hospitalizations or suicide attempts occurred in any study participants.

New and Planned Extramural or Intramural Research Initiatives

Pediatric HIV/AIDS

Because of medication advances and behavioral prevention, pediatric HIV/AIDS infection is rare in the United States. Transmission of HIV from mother to child has been reduced significantly, although not completely, as a result of pre- and postnatal medication administration. Infection among adolescents, particularly young racial/ethnic minority women and young men who have sex with men, continues to increase, signaling the need for further preventive efforts. For children born with HIV and for those who acquire the disease as children or adolescents, the use of antiretroviral therapy (ART) has prolonged life expectancy and quality; however, children with HIV/AIDS and their families face major issues in coping with the physical and psychological burden of HIV as they progress through childhood, adolescence, and young adulthood.

In FY 2001, NIMH supported more than 50 research projects with HIV-seropositive children and adolescents and prevention research for noninfected youths. The Institute supported the Adolescent Medicine Trials Network for HIV/AIDS Interventions (ATN) in collaboration with NICHD, NIDA, and NIAAA. ATN conducts primary, secondary, and tertiary prevention research in behavioral and therapeutic modalities with HIV-infected and at-risk youth ages 12–24 years.

Program Announcement (PA)

Suicide

NIMH and NIDA continue to receive applications in response to the March 2000 PA Interventions for Suicidal Youth, which will support efforts to develop and test interventions that build on both risk and protective factors for youth suicidal behavior. This PA identifies the need to test the effectiveness of interventions for reducing suicidal behavior for several approaches, ranging from broad-based community or school-based prevention efforts to more targeted approaches that reduce suicidal behavior in youth with identified mental disorders or substance use disorders.

Significant Ongoing Research Initiatives

Suicide

In late 1999, NIMH commissioned two comprehensive reviews of assessment instruments for suicidal behaviors and cognitions—one for children and one for adults. Both are now posted at www.nimh.nih.gov/research/suicide.cfm.

Recent NIMH initiatives have encouraged inclusion of more diverse samples of patients, including suicidal patients, in treatment research protocols. To help protect these patients and to encourage more researchers to include suicidal patients in research, NIMH conducted a workshop in June 2001, cosponsored by ORD and the American Foundation for Suicide Prevention, that involved experts in bioethics, law, and suicide treatment research to develop several documents describing approaches to consideration of the ethical issues in treating suicidal patients and ways to increase the safety and monitoring of suicidal patients in clinical trials. One document, *Issues to Consider in Intervention Research With Persons at High Risk for Suicidality*, is available on the NIMH Web site at www.nimh.nih.gov/research/highrisksuicide.cfm . Another document has been submitted to the journal: *IRB: A Review of Human Subjects Research*. A third document that includes case vignettes is being developed for publication. NIMH staff also have coordinated contacts among clinical researchers conducting trials to reduce suicidality to develop common research questions pertaining to competence to consent.

Workshops, Symposia, and Meetings

Eating Disorders: Workshop Update

In April 2000, NIMH sponsored the Prevention of Eating Disorders Roundtable to discuss the status of efforts regarding eating disorders, knowledge gaps, and future research activities to be considered, including the state of the science in understanding risk factors (e.g., neuroscience contributions), state of the science in effective preventive interventions for eating disorders and other relevant disorders (e.g., depression, substance abuse), and challenges to developing prevention efforts in eating disorders. A summary is available at www.nimh.nih.gov/research/edsummary.cfm in 2002 in the *International Journal of Eating Disorders*.

Suicide

NIMH, along with NIDA, NIAAA, the Substance Abuse and Mental Health Services Administration (SAMHSA), and the Centers for Disease Control and Prevention (CDC), is funding an Institute of Medicine study, Pathophysiology and Prevention of Adolescent and Adult Suicide. The report on research recommendations from this study is forthcoming.

NIMH, with cosponsorship from ORD, NCI, and NINR, convened a workshop in late spring 2002, Suicide Risk and Physical Illness, to review the research gaps and refine a research agenda.

NIMH awarded a conference grant (R13) to the University of Rochester in FY 2000 to conduct five annual meetings to review and develop scientific consensus on suicide risk factors and prevention strategies for several at-risk groups. These meetings have multiple NIH and other cosponsors. The first of these meetings, held in June 2001, focused on suicide prevention in adolescents and young adults. A second will focus on older adults.

Pediatric HIV/AIDS

The NIMH Center for Mental Health Research on AIDS and ORD cosponsored the meeting Pediatric AIDS and Mental Health Issues in the Era of ART, held September 10–11, 2001. Meeting participants identified and discussed the critical neurological, neuropsychological, psychosocial, and mental health issues of children and adolescents living with HIV/AIDS. A research initiative informed by recommendations from this meeting is being developed.

Additional Activities

Suicide

NIMH released a 2001 notice for Administrative Supplements for Human Postmortem Brain Research in Mental Illnesses. Several successful applications in response to this initiative focus on the neurobiology of depression in completed suicide victims.

NIMH staff are currently reviewing suicide prevention research priorities and are coordinating these efforts with NIDA and NIAAA, along with the CDC, to be responsive to HP2010 goals of reducing suicide deaths and attempts and responding to the national suicide prevention research objective described in the National Strategy for Suicide Prevention issued in May 2001.

National Center on Minority Health and Health Disparities (NCMHD)

Overview

NCMHD conducts and supports research, training, information dissemination, and other programs aimed at reducing the disproportionately high incidence and prevalence of disease, burden of illness, and mortality experienced by certain American populations, including racial and ethnic minorities and other groups (e.g., the urban and rural poor) with disparate health status.

For various reasons, such as genetics, health disparities in rare diseases among ethnic and racial minorities may be more pronounced than they are in common diseases among these groups. For example, conditions such as sickle cell anemia and keloid formation are linked to genes that are predominantly found within one racial group. Alternatively, for rare diseases such as systemic lupus erythematosis and breast cancer, which tend to occur across populations, there are still significant disparities in risk of disease development, severity of symptoms, and mortality. Indeed, racial and ethnic minorities and other underserved populations are likely to experience even greater barriers to the screening, diagnosis, and treatment of rare diseases than they are for common conditions because of various cultural, socioeconomic, and environmental factors.

NCMHD supports various rare disease projects in partnership with the other NIH Institutes and Centers. All projects involve a significant number of individuals from racial and ethnic populations and are aimed at increasing knowledge and, ultimately, eliminating health disparities. Several projects involve extensive community outreach and emphasize research training for minorities and research capacity building at minority-serving institutions.

NCI/NCMHD

Cancer

Cellular Responses to Mutagens in Lung Cancer: A Focus on Gender and Race

This intramural initiative seeks to determine whether mutagen sensitivity, p53 induction, and apoptosis in cultured lymphocytes will be predictive of lung cancer risk and whether this predictability varies by gender or race. Lung cancer is the most common cause of cancer-related deaths in African Americans and whites, and it has been proposed that African Americans have a higher risk than Caucasians for developing the disease, at a given level of smoking. Two hundred of each population group, with confirmed lung cancer, are being compared with two control groups, one hospital based and one population based, with a total of 400 of each of the two ethnicities.

Clinical and Molecular Correlative Studies in Minority Populations Involving Genitourinary and Gynecologic Malignancies

The purpose of this project is to assess the relationships among a series of molecular markers found in tumor tissue samples and defined clinical outcomes in African Americans and Hispanic Americans with genitourinary (e.g., prostate) or gynecologic malignancies. Three institutions are providing both research materials and investigators: Howard University, Louisiana State University, and the NCI Medicine Branch. Both minority and majority investigators are involved in this study.

Gene-Environment Interactions for Breast Cancer Risk and Survival in Different Racial and Ethnic Groups

This intramural project is explorating gene-environment interactions for breast cancer risk and survival in whites, African Americans, and Hispanics. The study will allow for a direct

comparison of risk factors for both initiation and aggressive disease.

Promotional Effects To Increase Participation in Case-Control Study of Renal Cell Cancer Among African Americans in the United States

The incidence of renal cell cancer rates among African Americans has increased rapidly compared with rates of other cancers. This population-based case-control study of renal cell cancer will be conducted in Detroit and Chicago. A total of 2,100 cases consisting of 1,400 whites and 700 African Americans, along with 2,800 control subjects consisting of 1,400 whites and 1,400 African Americans, will be recruited over a 4-year period. An important aspect of the project will focus on promoting the study to encourage participation among African Americans and provide incentives for participation.

NEI/NCMHD

Myopia

Myopia Development in Children

This study is an expansion of the Orinda Longitudinal Study of Myopia initiated in 1989, which focuses on an investigation of ethnicity and the development of myopia. Three new study populations have been added: African American, Asian American, and Hispanic American children. Although fewer than 2% of children beginning elementary school in the United States are nearsighted or myopic, the prevalence of myopia increases to more than 15% of middle school graduates and to 25% of the adult population.

NHLBI/NCMHD

Hypertension

Cellular Phenotypes of Salt Sensitivity

This study examines the role of cellular calcium turnover rate in salt-sensitive hypertension, which has a high prevalence in the African American population. Approximately 32 African Americans from the study population will be examined indepth as inpatients. A preliminary finding is that a male/female difference in blood pressure regulation is related to the menstrual cycle. Characterization of cellular calcium regulation in men and women on varying sodium intake levels is in progress. Ultimately, this information could be used as a predictor of the body's response to changing sodium diets.

Genetic Analysis of Human Hypertensive End-Stage Renal Disease (ESRD)

The focus of this initiative is a search, through various research approaches, for genes that predispose the African American population to hypertension-associated ESRD. NCMHD support has been targeted to add a sample of the Mexican American study population through support from a full-time recruiter. An additional piece of equipment will be purchased that will increase efficiency and handle the increased number of samples that generate genetic data.

Sarcoidosis

Sarcoidosis Genetic Linkage Consortium

The goal of this project is to identify sarcoidosis susceptibility genes and determine how these genes and environmental risk factors affect the manifestation of this systemic disease, of which African Americans have a higher incidence and experience a more severe form. NCMHD funds will increase the planned efforts of an epidemiologist, data coordinator, data entry person, and computer programmer to a level considered sufficient for successful completion of the study. Sarcoidosis is a disease of unknown origin, involving formation of granulomatous lesions especially in the lungs (for 90% of patients), liver, lymph nodes, and skin.

NIA/NCMHD

Alzheimer Disease

Enhancing Followup of African American Patients With Alzheimer Disease

The purpose of this program is to enhance research participation by African American Alzheimer patients and their caregivers. This is a collaborative effort between the University of California, Los Angeles and Charles R. Drew

University of Medicine and Science. The project seeks to resolve the issue of the low followup rate of African American patients after their first evaluation. This will be accomplished by 1) comparing an educational intervention with routine care to determine the impact of the intervention on followup participation, and 2) generating pilot data that can be used to construct a clinical trial of three interventions—education, low monetary, and high monetary—to determine how to best achieve followup participation.

Enhanced Participation by Medically Underserved African American and Hispanic Older People in Alzheimer Disease Research

The goal of this program is to increase participation by medically underserved elderly African Americans and Hispanics in Alzheimer disease research. This will be accomplished by increasing accessibility of the program to older individuals by encouraging support from neighborhood health centers, conducting telephone followups for all participants, and increasing the number of active participants in research projects.

NIAID/NCMHD

Asthma

Epidemiology of Home Allergens and Asthma

This is a longitudinal study of the association between socioeconomic disadvantage/minority status, allergen exposure, IgE antibody, and measures of asthma and wheezing, which will help determine why socioeconomically disadvantaged siblings may be at greater risk for allergy/asthma and determine how socioeconomically advantaged siblings may be protected.

The proposed project would extend investigation of risk factors for asthma development in older siblings via an ongoing birth cohort study. The parent grant currently supports limited studies on older siblings; however, research indicates an important relationship between cockroach allergen exposure and new-onset asthma. Cockroach allergen exposure in the home markedly enhanced the likelihood of developing new-onset asthma and recurrent wheezing in 4- to 5-year-old siblings who did not have asthma at the start of an extended observation period. In contrast, preliminary data suggest that elevated cat allergen levels protect against recurrent wheezing. Moreover, cockroach allergen exposure is a marker of socioeconomic disadvantage, and cat allergen is less common in low socioeconomic households. Data from this study will help determine whether

- High cockroach allergen exposure in early life is associated with increased incidence of allergy and asthma.
- Cat allergen exposure protects against asthma and allergy.
- Combinations of IgE antibody to allergens and high exposure to allergens in the bed predict wheezing and asthma severity.

NIAMS/NCMHD

Antiphospholipid Syndrome (APS)

APS Registry and Repository

The APS registry and repository is a multicenter, multispecialty, collaborative project that involves a group of leading APS investigators, including rheumatologists, hematologists, neurologists, and obstetricians at eight academic medical centers. This project will assist investigators in identifying basic immunological and genetic abnormalities, pathophysiological mechanisms contributing to thrombosis, fetal loss, and other clinical manifestations of APS. Additionally, the registry will be an important resource for projects aimed at improving methods of diagnosis and evaluating more effective and safer methods of treatment and prophylaxis. It will collect and update clinical, demographic, and laboratory data on patients with APS, asymptomatic patients with antiphospholipid antibodies, and individuals with serological and/or clinical features that fall within an expanded concept of APS.

Arthritis

Arthritis Among Latinos: A Study Based on National Data

This 1-year study involves a secondary analysis of data from the National Health Interview Survey. The goal of this study is to assist in uncovering underlying factors related to observed differences between Latinos and non-Latinos in prevalence rates of arthritis and associated levels of disability (both being greater in the Latino population). Ultimately, this information will be used to guide policy and intervention programs aimed toward reducing physical disability and improving health promotion in the Latino population.

Arthritis is an inflammation of the joints; however, the term arthritis is generally used to refer to more than 100 rheumatic diseases that can cause pain, redness, heat, swelling, stiffness, and decreased joint function. These diseases may affect parts of the body other than the joints, including connective tissues, muscles, tendons, ligaments, bones, and internal organs.

Registry and Repository of African Americans With Rheumatoid Arthritis

This is a collaborative project involving four major medical centers in the southeast United States to gather data that will be a resource for investigators interested in the genetics of rheumatoid arthritis in African Americans. The Consortium for the Longitudinal Evaluation of African Americans With Early Rheumatoid Arthritis registry identifies genetic and nongenetic prognostic factors of disease outcome with radiographic presence of bony erosions as the primary outcome measure at 3 years disease duration. The registry will be a basis for prospective analyses of factors predictive of the clinical phenotype and outcomes.

Keloids

Molecular Mechanisms for Keloid Formation

This project focuses on identifying the genes underlying susceptibility to keloids. The information will then be used to investigate the molecular basis for this abnormal wound healing that disproportionately affects African Americans.

Systemic Lupus Erythematosus (SLE)

Seven SLE studies are in progress. Three focus on identifying genes related to clinical manifestation of SLE with the hypothesis that the disease is polygenic. Four studies are examining related biochemical phenomena in SLE patients. One study of African American, Hispanic, and white patients is assessing relative influences of socioeconomic-demographic, behavioral-cultural, and immunogenetic factors in patients with SLE. The projects are

- Gene Mapping in Women With SLE.
- Genetics of Childhood Onset SLE.
- A Genetic Association With Lupus in American Blacks.
- Patient-Oriented Research: SLE.
- Accelerated Atherosclerosis in SLE: Prevalence Factors.
- Ectopic Germinal Center Reaction in SLE.
- Role of Nitric Oxide in SLE.
- Outcomes of SLE in Minorities: Nature vs. Nurture.

Genetic Association With Lupus in American Blacks

This prototypic inflammatory autoimmune disease SLE is a chronic, disabling disease that affects predominantly young premenopausal women and African Americans. African American women are afflicted 3–4 times more frequently than white women, and the risk of myocardial infarction in women with SLE is up to 50 times higher than expected in African American women ages 35–44.

This study aims to identify the genes that confer African American individuals with genetic susceptibility to SLE. The study will focus on

- Enlarging the collection of African American pedigrees multiplex for SLE.
- Establishing linkage at FcγRIIA and D1s3462 in African American pedigrees multiplex for SLE and pursuing the identity of the responsible genes.
- Establishing the presence of other links with SLE in African American pedigrees.
- Evaluating other phenotype definitions by examining their relationship with the links established in African American pedigrees multiplex for SLE.
- Exploring the criteria for SLE and the antinuclear antibody titer as quantitative trait phenotypes in African Americans with SLE.

Epidemiology of SLE and Cardiovascular Disease

This project will

- Compare the prevalence and extent of subclinical vascular disease in 200 SLE patients and matched control subjects.
- Determine whether the risk factors associated with subclinical vascular disease in patients with SLE are different from those in control subjects.
- Determine the risk factors associated with progression of carotid atherosclerosis in women with SLE over a 3-year period.

Epitopic Germinal Center Reaction in SLE

This study will test the hypothesis that an epitopic and accelerated germinal center reaction takes place in SLE.

Genetics of Childhood-Onset SLE

The goal of this initiative is to identify genes involved in the etiopathogenesis of human SLE and to characterize the mechanisms by which these genes influence disease development. Childhood-onset SLE represents a potentially unique subgroup of patients, because its early disease onset may be an indicator of increased genetic predisposition and penetrance and because childhood-onset disease is more severe than adult-onset disease in that the childhood form involves many organs and carries a worse prognosis. Thus, the project will study nuclear families of childhood-onset SLE subjects.

Lupus Multiplex Registry and Repository

The Lupus Multiplex Registry and Repository (LMRR) was established by NIAMS to accelerate progress in developing and identifying the genes and gene products related to SLE. It is a centralized repository of data, cells, and DNA of well-characterized multiplex SLE pedigrees. This is a valuable resource by virtue of recent advances in the ability to identify genes related to similarly complex autoimmune diseases such as insulin-dependent diabetes mellitus.

The following are important features of the LMRR:

- It is the largest collection of African American pedigrees multiplex for SLE.
- It has been used to demonstrate linkage for four genetic effects, but the gene is still unknown for each of these.
- It supports the work of five separate groups by providing data, DNA, plasma, and serum. Materials and data from 137 pedigrees (of which 46 are African American), containing 184 nuclear families and >800 family members and control subjects, are available for distribution.

The next phase of the project will focus on enlarging the available collections of multiplex pedigrees to include more African Americans, Native Americans, and Hispanic Americans.

Outcome of SLE in Minorities

This project examines the relative importance of socioeconomic-demographic, behavioral-cultural, and immunogenetic features in the clinical manifestations of SLE. The cohort will be increased from 229 to 450 patients to increase the ability to detect meaningful differences among the three ethnic groups. The project will also look at genes associated with SLE and the relationship among disease activity, disease damage, and self-perceived functioning in these patients and the factors that predict them.

Patient-Oriented Research

This project will conduct patient-oriented research and develop a mentor program for clinical investigators. The research plan will focus on the epidemiology of osteoporosis in women with SLE. This is a cross-sectional study to estimate the differences in bone mineral density between white and African American women and to determine the association of SLE risk factors with low bone mineral density. A longitudinal study will also be performed to follow the subjects entered in a cross-sectional study over a 2-year period to estimate differences in rate of bone loss.

Role of Nitric Oxide in SLE

Nitric oxide is a biologically pluripotent compound that is induced during immune responses and overproduced in mouse models of SLE. This study will look at nitric oxide production in human SLE patients compared with control subjects and the effects of nitric oxide on disease pathogenesis. This examination should provide insight into immune factors promoting nitric oxide production.

Scleroderma

Specialized Center of Research in Scleroderma

Scleroderma is a chronic hardening and thickening of the skin, which may manifest itself systemically as a disorder of connective tissue characterized by fibrotic degenerative changes in various body organs. NIAMS will begin its 5th year of study of scleroderma in minority populations.

The Choctaw Native American people in Oklahoma have a high prevalence of this disease. Many candidate genes have been ruled out, with evidence indicating a genetic association with fibrillin-1, osteonectin, and MHC. In addition, data from a multiethnic longitudinal study of patients with a disease duration of less than 5 years indicate potentially significant genetic, sociodemographic, and behavioral background factors among the groups studied.

Vitiligo

Mapping of Vitiligo Susceptibility Genes

The purpose of this initiative is to map vitiligo susceptibility genes. Although vitiligo affects all races, the effects are more significant in dark-skinned populations; therefore, it represents a major health disparity area of research for NIAMS. The study is designed to survey two independent populations and could potentially determine the genetic basis of vitiligo susceptibility and improve understanding of the pathologic process.

NIDDK/NCMHD

Diabetes Mellitus

Community Education for Gila River Indian Community

To provide the Gila River Indian Community with diabetes care, the Phoenix Epidemiology and Clinical Research branch has collaborated with the Gila River Health Care Corporation1 (a tribally run corporation that provides health care to the community) to develop plans to implement

- An enterprise health information system that supports patient registration and billing while integrating pharmacy, laboratory, and medical records information into a single electronic database that is easily accessible to health-care providers.
- A community-based diabetes care coordination program to improve the health of members of the Gila River Indian community who have diabetes by ensuring that they are seen by their primary care providers and by ancillary staff in accordance with recommendations and guidelines of the American Diabetes Association and the Indian Health Service.
- Supplemental funding for various health-related initiatives.
- A community-defined diabetes prevention program.

Community-Based Epidemiology Study of Type 2 Diabetes Mellitus in the Gila River Indian Community

Parallel to the current longitudinal population study of the Gila River Indian Community, a mobile and flexible health surveillance system will provide a brief examination and interview to assess diabetes, obesity, disability, and occupation of study participants. These data will facilitate assessment of how health is changing over time and how this behavior relates to employment and other health-related behaviors. The study will address important questions about the health effects of poverty and emergence from poverty through employment opportunities, enabling a nearly complete accounting of the health status of all members of the community.

Diabetes Research Training for Minority Students

This institutional research training program is designed to provide short-term training in biomedical research for four students from Howard University at either the Joslin Diabetes Center or the University of Pennsylvania Diabetes Education and Research Center. Mentors will give trainees 8–10 weeks' worth of research experiences and guide them through the research enterprise.

Precursors to Diabetes in Japanese American Youth

Approximately 450 Japanese American children (8–10 years old) will be studied to identify metabolic, lifestyle, and sociodemographic risk factors for type 2 diabetes in Japanese American youth. For comparison, 150 white children will also be evaluated. Based on studies of Japanese American adults, Japanese American children may be at high risk for developing type 2 diabetes because of a tendency toward central adiposity. However, little is known about the prevalence of type 2 diabetes in children, and the disease has not been systematically studied in children.

Family Investigation in Nephropathy of Diabetes (FIND)

FIND is a multicenter study with participating investigating centers at Harbor-UCLA Medical Center, University of California, University of New Mexico, Case Western Reserve University, University of Texas at San Antonio, Wake Forest University, Johns Hopkins University, and the NIDDK Phoenix group. The purpose of this study is to identify genetic loci that confer susceptibility to or protection from nephropathy in patients with diabetes mellitus. The major investigation will be family study of concordant and discordant sib pairs. A second study will use a MALD (mapping by admixture linkage disequilibrium)-based analysis strategy to investigate the difference between susceptibility and protective loci in different populations with varying risks of nephropathy—for example, African American and Hispanic American compared with white patients.

Gestational Diabetes Component of Diabetes Prevention Program

A total of 3,234 subjects have been/are enrolled in a three-arm study with two active treatment groups (medication and lifestyle). Of these subjects, 68% are women, and 165 of these women currently have or have had gestational diabetes (GDM). Almost 50% of participants are from minority groups. Funds from NCMHD have provided significant recruitment and retention resources to randomize women, those with GDM, and minorities into the program. The high level of compliance by volunteers will enhance staff ability to assess prevention and intervention strategies.

Look AHEAD: Action for Health in Diabetes Sustained Support for Enhanced Recruitment/Retention Efforts

Look AHEAD: Action for Health in Diabetes (formerly called SHOW), supported in conjunction with NHLB, NINR, ORWH, and CDC, studies the health impact of interventions designed to produce and sustain weight loss over the long term in obese individuals with type 2 diabetes. Because obesity disproportionately affects minority populations, especially women,

studies of obesity treatment are of particular interest to minority health. The grantee's intramural research group in Phoenix will establish a Southwest American Indian Center to recruit study participants from Native American populations in Arizona and New Mexico. Five thousand obese individuals at 16 clinical centers across the United States will be studied over a 2.5-year period.

National Diabetes Education Program

This project extends community-based outreach initiatives to promote current National Diabetes Education Program campaign messages and implement appropriate interventions that promote behavior and lifestyle changes for people with diabetes. This extended outreach effort will target minority audiences and communities.

Prevalence and Progression of Type 2 Diabetes Risk in Mexican American Youth

An estimated 2,000–4,000 Hispanic American fourth graders in the San Antonio, Texas, school district will be studied to investigate prevalence rates of diabetes and impaired fasting glucose. A representative sample of Mexican American children will be screened for various risk factors, hyperinsulinism, impaired fasting glucose, and diabetes. In addition, a longitudinal followup of a subset (approximately 300 children) of the population screened will be used to describe the natural history of children with identified metabolic abnormalities and determine whether easily performed screening tests can be used to predict the risk of developing diabetes.

Hepatitis C

Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis Trial—Enhanced Minority Recruitment

Hepatitis C is an inflammation of the liver caused by one of three known viruses with similar effects. Because some minority subpopulations are at a higher risk than others for hepatitis C, enhanced recruitment for a cirrhosis treatment study was warranted. Of the total recruitment goal of 1,350 patients, 20% of them from racial minorities, 222 study participants have been enrolled, with 22% minority patients. Contacting minority physicians at the study sites for potential enrollees has contributed to the improved minority enrollment.

Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C (Virahep-C)

Eight geographically distributed clinical centers, a data coordinating center, and three or four ancillary studies will be supported to examine particular issues surrounding viral resistance to interferon in hepatitis C. The 400 study participants will include approximately equal numbers of African Americans and non-Hispanic whites, all of whom have chronic hepatitis C and are infected with HCV genotype 1.

Kidney Disease

Study of African Americans With Kidney Disease and Hypertension

This multicenter clinical trial is investigating treatment interventions to slow the progression of kidney disease in African Americans with hypertension. A total of 1,094 patients were randomized to one of three antihypertensive medications. Patients taking the angiotension-converting enzyme inhibitor ramipril were less likely to have progressive worsening of kidney function than those on the calcium-channel blocker, which was therefore eliminated. NCMHD has substantially supported the trial since FY 1992 and provides ongoing support for the three minority-majority collaborations in the full-scale trial and in the Howard University Center.

Prospective Cohort Study of Chronic Renal Insufficiency

This project establishes clinical centers and a data coordinating center to conduct a 7-year prospective cohort study of approximately 3,000 patients with chronic renal insufficiency. The two major goals of this study are 1) to determine the risk factors for accelerated decline in renal function, and 2) to determine the incidence and identify risk factors for cardiovascular disease. A major emphasis will be to determine the causes of the increased burden of renal disease in minority populations. Approximately half of

the cohort will consist of individuals from racial and ethnic groups.

Prostatitis

NIDDK Chronic Prostatitis Collaborative Research Network

The Chronic Prostatitis Collaborative Research Network is a consortium of six recruitment sites and a data coordinating center developed in Jackson, Mississippi, to study the epidemiology of chronic abacterial prostatitis. Funding supports the continued operation of this cohort study and focuses recruitment on African American men.

Sickle Cell Disease (SCD)

Secondary Hemochromatosis in β-Thalassemia and SCD

The purpose of this study is to determine whether the pathological effects of iron overload secondary to hypertransfusion are different in SCD and β-thalassemia. This is a particularly important issue with the change in guidelines for early transfusion as a part of clinical management of SCD in selected pediatric patients at risk for stroke. Iron-related organ injury and death are common in patients with β-thalassemia, but, to date, similar organ pathology and mortality have not been reported in SCD after hypertransfusion. The primary hypothesis of this study is that hypertransfused patients with SCD show less organ damage than patients with β-thalassemia. However, it is critical to examine the organ damage and cellular distribution of iron in hypertransfused patients with β-thalassemia and SCD and to determine whether severe organ damage occurs less frequently in hypertransfused patients with SCD than in patients with β-thalassemia. An attempt will be made to evaluate whether markers for early organ dysfunction can be identified and used to guide chelation therapy in SCD patients.

Syndrome X

Genetic Basis of Syndrome X on the Island of Kosrae

A series of genetic and clinical studies will examine the increased incidence of obesity, hypertension, diabetes, and dyslipidemia on the Pacific Island of Kosrae. The current project augments studies completed in 1994 on 2,364 Kosraeans over age 20—nearly the entire adult population. This project will apply modern methods in human genetics to map the genes that cause the components of Syndrome X. In addition, a followup clinical study will be performed on the entire island population over age 16. Approximately 1,000 new participants are expected to be added to the previous study population.

NIEHS/NCMHD

SLE

Hormonal and Environmental Risk Factors for SLE: The Carolina Lupus Study

This is the first population-based epidemiological study of SLE in the United States. It provides the opportunity to examine occupational and environmental risk factors in a previously understudied population. Efforts may help to illuminate etiologic pathways and to develop targeted preventive measures. Of the subjects in the study, 60% are African American.

National Institute of Neurological Disorders and Stroke (NINDS)

Overview

The mission of NINDS is to reduce the burden of neurological disease—a burden borne by every age group and every segment of society worldwide. The brain, spinal cord, and nerves are vulnerable to hundreds of disorders, most of them individually rare. Even common disease categories, such as stroke, epilepsy, and Parkinson disease, include rare subtypes. Therefore, research on rare diseases is a major aspect of NINDS activities.

Recent Scientific Advances

The following examples are a sampling of recent NINDS-supported research advances related to rare diseases.

Alexander Disease

Alexander disease is a rare form of leukodystrophy that, in its most serious form, is fatal in early childhood. The leukodystrophies disrupt normal electrical activity in the nervous system by their effects on the white matter, which takes its appearance from the sheathes that cover long nerve fibers in the brain and spinal cord. Basic scientists studying the cytoskeleton developed a strain of genetically engineered mice that overproduce the protein glial fibrillary acidic protein (GFAP). Mice that produced too much GFAP died young, and their glia contained abnormal protein aggregates reminiscent of those in brain cells of children who die from Alexander disease. Subsequent study of DNA samples from patients with Alexander disease confirmed that mutations in the gene for GFAP are responsible for most cases of the disease.

Amyotrophic Lateral Sclerosis (ALS)

ALS, or Lou Gehrig disease, progressively destroys the nerve cells that control voluntary movement, ultimately leading to paralysis and death. Perhaps 10% of ALS is inherited, and the cause of most cases is not known. About a decade ago, scientists discovered that defects in the gene *SOD1* are responsible for about 20% of the inherited cases of ALS. That discovery led to development of a mouse model of the disease that has been a boon in efforts to try to understand the disease and develop treatments. This year, scientists developed a rat SOD1 model of ALS that should be a valuable resource for experiments that are difficult or impossible in mice. Researchers also identified a gene defect responsible for a rare juvenile form of ALS, which should lead to further insights about the disease.

Ataxia

Ataxia refers to a loss in the ability to coordinate movement. Ataxias may be inherited as degenerative diseases, result from developmental brain malformations, or be acquired from trauma, infections, vascular events, neoplasms, demyelination, metabolic disorders, or toxins. The many inherited ataxias have long defied attempts at rational diagnosis and classification. However, in recent years, identification of more than a dozen different genes that, when defective, can cause these movement disorders is leading to improvements in diagnosis and understanding. A new study examining patients with unexplained ataxia found cases associated with defects in coenzyme Q10, which is a crucial component in cell conversion of food to useful energy. Once identified, patients responded to dietary supplementation of coenzyme Q10 with improved ataxia, increased strength, and less frequent seizures.

Creutzfeldt-Jakob Disease (CJD)

CJD is a devastating neurodegenerative disorder with no known treatment. CJD is in the same class of transmissible spongiform encephalopathies (TSEs) that includes bovine spongiform encephalopathy (BSE). Rogue proteins called prions appear to cause these disorders by forming harmful aggregates in the brain; therefore, researchers developed a cell culture test for

screening drugs that might prevent or clear the abnormal prion aggregates. Two drugs that proved effective in these cell culture tests are already approved for other medical uses: chlopromazine, used for the treatment of schizophrenia, and quinacrine (or atabrine), widely used during World War II for malaria. Testing of these compounds in people with vCJD, the form of disease linked to BSE, is under way in the United Kingdom. NINDS is also playing a major role in coordinating the NIH component of the Department of Health and Human Services TSE/BSE Action Plan.

Fabry Disease

Fabry disease is the second most prevalent hereditary metabolic storage disorder. In storage diseases, a faulty enzyme allows certain substances to accumulate to harmful levels. In Fabry disease, symptoms typically first appear during childhood or adolescence with recurrent episodes of severe pain in the hands and feet, skin lesions, and damage to the cornea. The disease usually progresses to cause death through effects on the kidneys, heart, or blood vessels of the brain. Building on many years of preliminary research, a NINDS intramural research team has completed a randomized, controlled phase II clinical trial to test the effectiveness of replacing α-galactosidase, the missing enzyme in Fabry disease. The therapy reduced the level of severe pain, improved pain-related quality of life, and appeared to reduce kidney problems and improve heart function.

Familial Dysautonomia

Familial dysautonomia, also known as Riley-Day syndrome or hereditary sensory and autonomic neuropathy type III, is an invariably fatal inherited disorder that affects sensory nerve cells and the autonomic nervous system. The wide range of symptoms reflects dysfunction of sensory, gastrointestinal, respiratory, and cardiovascular systems normally regulated by these nerve cells. Two independent teams of scientists have determined that the disease is caused by defects in the IκB kinase complex-associated protein gene *(IKBKAP),* which normally regulates the activity of other genes. The findings immediately provide the basis for carrier screening in the Ashkenazi Jewish population, where the mutation is most common and in the long run will lead to better understanding of the disorder. In particular, if scientists can determine why some types of cells, but not nerve cells, can make a functional IKBKAP protein despite the mutation, as the studies showed, they may be able to suggest strategies for treatment.

Huntington Disease

Huntington disease is an inherited disorder that causes cognitive and motor difficulties, including chorea. Symptoms usually begin in early to mid-adulthood and progressively worsen, leading to death. An inherited defect in a protein called huntingtin causes the disorder. This year, the largest clinical trial for the disease found that the drugs coenzyme Q10 and remacemide did not slow the progression of the disease, but it did provide clues for future studies. In other work, scientists are making progress in understanding how huntingtin causes the disease. A new study in mice genetically engineered to have a Huntington disease-like disorder showed that cystamine reduced tremors and prolonged the life of the mice. This drug and others are moving toward testing in people with this disease.

Myotonic Dystrophy Type 2

Myotonic dystrophy, which is the most common adult form of muscular dystrophy, causes a characteristic pattern of muscle loss, myotonia, and effects on the heart, eyes, and hormones. In 1992, scientists discovered the gene defect that causes one subtype of myotonic dystrophy, although exactly how this mutation produces the complex symptoms of the disease is still not clear. Now researchers have determined what causes the second form of myotonic dystrophy. The defect is in the zinc finger protein 9 gene *(ZNF9)*, because it codes for a protein with a structure called a zinc finger that normally recognizes and binds to DNA. The finding should allow development of a genetic diagnostic test and provides important clues to the causes of myotonic dystrophy.

Neurofibromatosis

Neurofibromatosis type 1 (NF1) is a genetic disorder that affects the growth of nerve cells, causing neurofibromas, skin changes, and bone deformities. About half of the people with the disorder also experience cognitive disabilities. Researchers working with mice genetically engineered to develop an NF1-like disorder have traced the learning problems to excessive activity of a crucial signaling molecule called Ras. They found that they could improve learning in the NF1 mice by using an experimental drug that reduces the Ras signaling. Studies are now revealing whether these findings can explain the cognitive problems in people with NF1 and lead to potential treatments.

Stiff Person Syndrome (SPS)

SPS is characterized by muscle rigidity and heightened sensitivity to noise, touch, and emotional distress that can set off muscle spasms. People with this disorder are often too disabled to walk or are afraid to leave the house because of stimuli-triggered muscle spasms and frequent falls. Researchers previously implicated an immune response that affects a neurotransmitter that helps the brain and spinal cord control movement. An NINDS intramural study found that intravenous immunoglobulin administered to patients suffering from SPS gives dramatic relief from disabling symptoms

Tourette Syndrome

A clinical trial jointly funded by NINDS and the Tourette Syndrome Association showed that a nicotine patch helps control the symptoms of Tourette syndrome in children and adolescents. Use of the patch allowed physicians to control symptoms, including motor tics, with much lower doses of the drugs normally used to treat the disorder. These drugs, also used as antipsychotics, often have strong unwanted effects on motor control and thinking, so reducing doses is important. Nicotine dependence was not evident in this study, but scientists are looking for other drugs that can mimic the effects of nicotine without its addictive risks and side effects.

Research Initiatives

NINDS relies heavily on the insight and ingenuity of individual scientists to seek out opportunities for research progress. Unsolicited investigator-initiated grants comprise the major share of NINDS work on rare disorders, as they do for more common diseases. The Institute also issues solicitations of various types to stimulate research when appropriate. In FY 2001, NINDS, often in cooperation with other components of NIH, issued or awarded grants responding to several solicitations relevant to rare disorders. These include

- Spinal Muscular Atrophy, Amyotrophic Lateral Sclerosis, and Other Motor Neuron Disorders.
- Exploratory Grant in Pediatric Brain Disorders: Integrating the Science (includes Friedreich ataxia, ataxia telangiectasia, Batten disease, Duchenne muscular dystrophy, fragile X syndrome, Sturge-Weber syndrome, holoprosencephaly, and many others) (with NICHD and NIMH).
- Research on Facioscapulohumeral Dystrophy (with NIAMS).
- Development of Innovative Treatment Approaches to Autism (includes Rett syndrome) (with NIMH, NICHD, and NIDCD).
- Therapeutic and Pathogenic Approaches for the Muscular Dystrophies (with NIAMS).
- Research on Autism and Autism Spectrum Disorders (includes Rett syndrome) (with NIDCD, NIMH, NICHD, and NIEHS).
- Gene Discovery for Neurological and Neurobehavioral Disorders.
- NINDS Administrative Supplements: FDA-Approved Compound (with ALS Association, Hereditary Disease Foundation, and Huntington Disease Society of America).
- Gene Therapy for Neurological Disorders.

NINDS is also encouraging research on stem cells in the nervous system, which may be relevant to many rare neurological disorders. Among recent efforts in this area are

- Short-Term Courses in Human Embryonic Stem Cell Culture Techniques.
- NINDS Administrative Supplements for Research on Human Embryonic Stem Cells.
- Plasticity of Human Stem Cells in the Nervous System (with other ICs).

Workshops, Symposia, and Meetings

In FY 2001, NINDS led or participated in several workshops relevant to rare diseases. In most cases, the Institute collaborated with ORD or other appropriate components of NIH and often with private disease-related groups. Among these meetings were

- Gene Therapy for Neurological Disorders.
- From Gene to Function in Dystonia.
- Hypertonic Movement Disorders Workshop.
- First Scientific Workshop of Hallervorden-Spatz Syndrome.
- Strategies for Therapy of MPS and Related Diseases.
- 2001 CAG Triplet Repeat Disorders, a Gordon Conference.
- Mucolipin, TRPs and Human Disease.
- Workshop on Research Opportunities on Human Neuroborreliosis.

For FY 2002, NINDS is working with ORD on several meetings, including those focused on research and clinical practice in Joubert syndrome, pediatric neurotransmitter diseases, brain banking, familial dysautonomia, tuberous sclerosis complex, childhood motor disorders, and new therapeutics in skeletal and central nervous systems, with applications to mucopolysaccharidosis.

National Institute of Nursing Research (NINR)

Overview

NINR supports clinical and basic research to establish a scientific basis for the care of individuals across the life span, from management of patients during illness and recovery to reduction of risks for disease and disability, promotion of healthy lifestyles, promotion of quality of life in those with chronic illness, and provision of care for individuals at the end of life. NINR's rare diseases research portfolio investigates strategies to control, manage, and prevent biobehavioral complications of such pathologies.

Recent Scientific Advances

Childhood Acute Lymphoblastic Leukemia (ALL)

NINR-funded research is examining the social-emotional functioning, psychomotor skills, and cognitive abilities of children diagnosed with ALL who receive whole-brain radiation, intrathecal chemotherapy, and high-dose systemic chemotherapy. Published findings suggest that neuropsychological outcome and declines are related to both demographic and treatment characteristics, depending on the cognitive domain examined. To determine whether prophylactic central nervous system chemotherapy for childhood acute lymphoblastic leukemia is associated with declines in neuropsychological abilities, growth curve analysis was used to examine neuropsychological outcome and treatment-related change in children who were treated at two childhood cancer centers. A comprehensive test battery was administered at baseline and slightly more than 4 years postdiagnosis (mean age at diagnosis, 6 years 1 month). Results indicated modest declines in arithmetic, visual-motor integration, and verbal fluency. Intrathecal and systemic treatment was related to poorer visual-motor integration at 4 years postdiagnosis and a faster rate of decline in visual-motor integration skills across the observation period than intrathecal treatment alone. Arithmetic proficiency at 4 years postdiagnosis was related to maternal education, but the rate of decline was not. Verbal fluency was unrelated to demographic or treatment variables.

Acute Respiratory Distress Syndrome (ARDS)

NINR researchers are studying optimum ways to wean patients diagnosed with ARDS from mechanical ventilation and have published some of their findings. One study evaluated heart rate variability (HRV) and thermodynamics to determine the effect of HRV on weaning from mechanical ventilation. A canine model was used, with control animals having normal cardiac function during baseline controlled mechanical ventilation. The experimental group was exposed to high mechanical ventilator pressure settings to simulate parameters required when treating patients with ARDS. Hemodynamic responses measured in both groups included cardiac output, heart rate, and right-ventricular end-diastolic volume. Alterations in HRV were associated with significantly increased heart rate and reduced right-ventricular end-diastolic volume, indicating increased sympathetic and decreased parasympathetic tone on exposure to high ventilator pressures. Patients requiring mechanical ventilation when diagnosed with ARDS often fail to wean from mechanical ventilation, possibly because of HRV dysfunction. NINR-funded investigators anticipate that the conclusions of this study will provide insight into methods of optimizing cardiovascular and respiratory function to modulate HRV in ARDS patients and improve success in weaning from mechanical ventilation.

Another NINR-funded prospective randomized study is investigating the effect of early and repeated prone positioning on clinical outcomes in pediatric ARDS patients who require mechanical ventilation. Early reports indicate improvements in oxygenation without serious iatrogenic injury after prone positioning.

Multiple Sclerosis

NINR researchers report that health-promotion practices to enhance physical activity, nutrition, and stress management in women with multiple sclerosis can increase quality of life and years of healthy life. Disease-modifying agents that decrease the accumulation of disability are costly and inconvenient and may have significant side effects. The findings suggest that much less costly health-promotion interventions may hold substantial promise as a complementary strategy for decreasing the burden of functional limitations.

Irritable Bowel Syndrome (IBS)

NINR researchers reported an association between autonomic nervous system (ANS) function and gastrointestinal symptoms in women with IBS. ANS balance was assessed in women with and without IBS via laboratory tests of function (i.e., expiratory/inspiratory ratio, Valsalva, posture changes, and cold pressor) and spectral and nonspectral HRV measures. Women with and without IBS were recruited and interviewed, and then completed a laboratory assessment and wore a 24-hour Holter monitor. Analysis with the entire sample showed little difference between IBS and control women and between subgroups with IBS on either laboratory measures or HRV measures. However, analysis restricted to women with severe IBS symptoms showed pronounced differences between two IBS subgroups on HRV measures. Parasympathetic tone was significantly lower and ANS balance was significantly higher in the constipation-predominant than the diarrhea-predominant group. These findings point out the importance of considering symptom severity when interpreting studies of IBS.

Narcolepsy

NINR researchers report that scheduled sleep periods are helpful for narcoleptic patients who remain profoundly sleepy despite treatment with medications. The research objective was to determine whether the combination of scheduled sleep periods and stimulant medications was more effective than stimulant medications alone in controlling the excessive daytime sleepiness experienced by narcoleptic patients. Narcoleptic subjects were randomly assigned to one of three treatment groups: two 15-minute naps per day, a regular schedule for nocturnal sleep, or a combination of scheduled naps and regular bedtimes. Measures of symptom severity and unscheduled daytime sleep were obtained at baseline and at the end of the 2-week treatment period, using the Narcolepsy Symptom Status Questionnaire (NSSQ) and 24-hour ambulatory polysomnographic monitoring. No alterations were made in stimulant medications during the study period. The results revealed that addition of two 15-minute naps did not alter symptom severity or the duration of unscheduled daytime sleep. Regular times for nocturnal sleep reduced perceived symptom severity but did not reduce the amount of unscheduled daytime sleep. Only the combination of scheduled naps and regular nocturnal sleep times significantly reduced both symptom severity and the amount of unscheduled daytime sleep in treated narcoleptic subjects. The type of sleep schedule prescribed, however, was less important than the severity of the patients' pretreatment daytime sleepiness. Subjects with severe daytime sleepiness benefited from the addition of scheduled sleep periods, whereas those who were only moderately sleepy or able to maintain alertness did not benefit from scheduled sleep periods.

Epilepsy

Most health-care providers report anecdotally that a camping experience helps children and adolescents with chronic health conditions to develop more positive attitudes toward their condition. However, children's and adolescents' perceptions have rarely been studied systematically. This pilot study examined children ages 8–16 at camp to assess the effect of a camp experience on their attitude toward epilepsy. Attitudes, measured by the 13-item Child Attitude Toward Illness Scale (CATIS), were assessed before and after the camp experience. No pre- or posttest difference in attitude toward epilepsy was found in the total group. However, when attitudes were examined by seizure frequency, there was a trend for those with more frequent seizures to report a more positive attitude after the camp experience.

Research Initiatives

Program Activities

NINR sponsored three rare disease research workshops in FY 2001. One workshop, Increasing Nursing Postdoctoral Opportunities in Cystic Fibrosis, was held May 1–2, 2001. Its purpose was to identify ways to increase the number of nurse researchers engaged in cystic fibrosis (CF) research. Potential research areas included psychosocial needs of children and their families; exercise and nutrition plans to promote healthy lifestyles; questions surrounding the symptom management of the biology, genetics, and physiology of CF; ethical questions of genetic testing; and use of technology to support self-care and home care.

A second workshop, Research in Informal Caregiving, was held July 26–27, 2001. Its purpose was to evaluate the caregiving literature in regard to patients diagnosed with rare diseases and to identify gaps in the knowledge base for care of these patients across the life span. The state of caregiving science was considered for several potential research areas of opportunity, including chronic illness, aging, technology dependence, cognitive impairment, under-studied cultural and ethnic diversity groups, caregiver support systems, measurement of caregiving and its outcomes, and cost issues.

The third workshop, End-of-Life in Genetic Illness, was held September 26, 2001, with the goal of bringing together researchers, clinicians, and people living with genetic illnesses to discuss issues related to the end of life in genetic illnesses. Highlighted areas for research unique to this subject included communication and the wording of messages by health-care providers, balancing hope with the realities of the genetic diagnosis, and guilt, fear, and remorse among family members at the possibility of having passed on a life-threatening disease.

National Center for Research Resources (NCRR)

Overview

NCRR develops and supports critical research technologies that underpin health-related research to maintain and improve the health of citizens of the United States. NCRR supports shared resources, sophisticated instrumentation and technology, animal models for study of human disease, clinical research, and research capacity building for underrepresented groups.

Through its support of multidisciplinary research, NCRR is uniquely positioned to provide either primary research support or resource support in partnership with other Institutes or Centers to address emerging clinical and basic research needs, such as the study of rare diseases. Expansion of NCRR's efforts in new biotechnologies and instrumentation, development of animal models, and clinical research will foster interdisciplinary collaborations and advance NIH's efforts to study rare diseases.

Biomedical Engineering and Instrumentation

Glioblastoma

Treatment in which an inactive agent is rendered potent by light exposure is known as photodynamic therapy (PDT). This therapy, involving delivery of an unreactive drug to a tumor and then using light to activate the drug so that it can kill cancerous cells, is being developed for a rare brain tumor, glioblastoma multiforme. A problem with using PDT in the brain is that light does not penetrate brain tissue effectively; consequently, long treatment times are required. In this study, investigators at the University of California, Irvine, with support from NCRR, report on use of this sophisticated technology to deliver long-duration PDT. The results of preliminary studies support the development of an indwelling balloon applicator for delivery of light doses in long-term multifractionated PDT regimens.

Cystic Fibrosis (CF)

Despite optimal medical care, secondary problems associated with CF can adversely influence calcium status and bone development, resulting in progressive loss of bone strength leading to bone fracture in these patients. Alterations in calcium metabolism and deficits in both attained height and bone mineral density are frequently reported in individuals with CF, although the causes are difficult to elucidate because of the many nutritional, hormonal, and immunological influences on bone turnover.

Because low bone mass is largely an irreversible process, focus should be placed on optimal bone development and attainment of peak bone mass in children affected with this disease. To address these issues, a study is being undertaken that will investigate aspects of calcium metabolism in a group of girls with CF. This study, in which calcium absorption and bone calcium turnover are directly examined, was made possible through use of modern technologies with stable isotopes of calcium in a technique known as thermal ionization mass spectrometry, available at an NCRR-supported laboratory at the Johns Hopkins School of Public Health. All values are compared with data obtained in girls without CF, matched for stage of sexual maturity, who have participated in similar studies of calcium metabolism. Other indicators of bone status, including bone mineral density, indirect markers of bone formation and resorption and hormones involved in calcium metabolism will be assessed in this sample of girls and compared with the control subjects. Understanding how calcium is metabolized in subjects with CF will help ascertain whether promoting calcium supplements is likely to benefit these patients or whether deficits in other metabolic pathways, such as calcium absorption or bone resorption, may make supplements ineffective.

Comparative Medicine

Parkinson Disease (PD)

PD, which primarily affects the elderly, can be defined by stages. At the onset of clinical symptoms, tremor, limb stiffness, slowness of movement, and gait disturbances appear but do not interfere with daily life. Advanced stages may be characterized by sufficient disability to require assisted care. Current drug therapies are largely designed to replace the essential brain chemical dopamine, which is produced in insufficient quantities in PD patients. Scientists at the NCRR-supported New England National Primate Research Center are testing and systematically evaluating promising drug candidates that enhance dopamine activity in a monkey model of PD. The ability to identify and objectively assess drug efficacy in an animal model of both early and late stages of the disease will expedite development of new effective therapeutics.

Clinical Research

Fabry Disease

Fabry disease results from a genetic error wherein deficiency of an enzyme known as α-galactosidase (α-Gal-A) leads to disruption of the metabolism of body substances known as glycolipids. Because these products cannot be broken down, they instead accumulate predominantly in the kidney, heart, and skin, resulting in pain, major organ failure, and usually premature death. A way of normalizing the level of the missing enzyme has long been sought.

In a multicenter study of 58 patients, supported in part by the General Clinical Research Centers at Mt. Sinai School of Medicine and at Cedars Sinai Medical Center, Fabry patients were treated every 2 weeks for 20 weeks with either a genetically engineered form of the missing enzyme (α-Gal-A) or with placebo. Neither the doctors nor the patients knew which patients received which substance (double-blind study). Thereafter, in an extension study, all patients were knowingly and openly treated with recombinant α-Gal-A. Efficacy was primarily judged by the percentage of patients in whom reduction to normal or near-normal levels in microvascular deposits of the complex phospholipids in kidney, heart, and skin were demonstrated. Changes in levels of pain and quality of life were also monitored.

The results showed that, in the double-blind study, 20 of 29 patients (69%) in the recombinant α-Gal-A group had no microvascular endothelial deposits in the kidneys, heart, or skin of the complex phospholipid after 20 weeks of treatment, compared to none of the 29 patients in the placebo group. This difference was biostatistically significant. Plasma levels of the phospholipid were directly correlated with clearance of the microvascular deposits. After 6 months of open-label therapy, all patients in the former placebo group and 98% of patients in the α-Gal-A group showed clearance of the complex phospholipid. Only minor side effects (e.g., chills and fever) were noted from the treatment. This study thus demonstrates the efficacy of and tolerance to recombinant α-Gal-A in the treatment of the clinical symptoms of Fabry disease.

Porphyria Cutanea Tarda (PCT)

PCT is a disease characterized by skin fragility, blisters, and thickened skin on sun-exposed areas. Occurring in 1–5 per 25,000 individuals, one-third of these patients have the familial form (F-PCT) stemming from a gene defect due to an abnormality in the enzyme uroporphyrinogen decarboxylase (URO-D). Symptoms are accentuated when the patient is challenged with additional factors that further reduce the URO-D activity in the liver.

This enzyme is needed to convert uroporphyrinogen, a byproduct of the heme portion of the red blood cells, into harmless compounds. Deficient levels (or abnormal function) of URO-D allow uroporphyrinogen to accumulate in the liver and skin and cause the symptoms of PCT.

Investigators at the University of Utah General Clinical Research Center (GCRC) collected blood cells from 10 F-PCT patients and identified and then sequenced the abnormal gene in each individual. Using sophisticated molecular methods and x-ray crystallography available

within the GCRC core laboratory, they determined the structure of the enzyme produced by various changes (mutations) in the gene. These studies demonstrated that the delicate cleft existing in normal URO-D was abolished by the single amino acid change in the abnormal gene. This seemingly minor abnormality made the enzyme nonfunctional, predisposing the patient to accumulate uroporphyrinogen and develop PCT symptoms. Understanding the genetic basis of how their abnormal products function will facilitate development of medical therapies for both the inherited and noninherited forms of this genetic disorder.

Myotonia

Myotonia congenita is an inheritable disorder characterized by slow relaxation of muscles. This is exemplified by a handshake in which the affected individual is only very slowly able to open and disengage his hand. Early symptoms may include gagging and difficulty in swallowing because muscles in the throat are slow to relax. The disease process is thought to involve an abnormality in the flow of charged molecules across the cell membranes of muscles, causing continuous stimulation of the muscle to contract despite a call from the nervous system for the muscle to relax.

Funded by NCRR, researchers at the University of Nevada, Reno, are studying genetic mutations in the channels that allow negatively charged molecules such as chloride ions to flow abnormally across the membranes of muscle cells. They will use a combination of experimental approaches, including human molecular genetics and the selective breeding of mice, to display this defect. This research may lead to new treatments not only for myotonia but also for cardiovascular diseases such as irregular heartbeat. More information on this research can be found at www.unr.edu/med/dept/pharmacology/COBRE.

Von Willebrand Disease (VWD)

VWD is a hereditary bleeding disorder caused by a deficiency of a blood substance known as von Willebrand factor (VWF). VWF helps normal blood clotting by influencing platelets to adhere to the blood vessel wall and to each other. Bleeding associated with this disorder is mild under most circumstances; if a person with VWD is injured or in need of surgery, techniques are available for elevating their levels of VWF. However, some subtypes of VWD do not respond to these treatments; these subtypes warrant further study.

NCRR supports the Molecular Genetics of Blood Disorders Research Program at the University of Puerto Rico Medical Sciences Campus, where both basic and clinical researchers study VWD. The long-range goal of the research is to determine the genetic mutations causing this blood disorder in the Puerto Rican population. This knowledge may lead to novel therapies, especially for the rare disease types that currently have no form of treatment.

Usher Syndrome

Usher syndrome is the most common condition that involves both hearing and vision problems. The major symptoms of Usher syndrome are impairment of hearing and sometimes balance and an eye disorder called retinitis pigmentosa, in which vision worsens over time. More than half of the estimated 16,000 deaf-blind people in the United States are believed to have Usher syndrome. Currently, no treatment exists for this inherited disorder.

NCRR-funded researchers at West Virginia University are using a broad range of techniques (e.g., genetic analysis and functional brain imaging) to study sensory disorders that are inherited or that occur during development. They will combine anatomical, physiological, and molecular studies of developing nerve cells to determine the mechanisms behind this defect. This work may lead to a treatment strategy. More information on this research can be found at www.hsc.wvu.edu/snrc.

National Library of Medicine (NLM)

Overview

NLM provides information resources useful to rare disease research and to those seeking information about conditions that affect them or their families.

Database Resources

Articles on rare diseases that have long been available in the MEDLINE database are now accessible to researchers, health professionals, and the public through NLM's free Web-based PubMed system (www.ncbi.nlm.nih.gov/) and the TOXNET system (toxnet.nlm.nih.gov). MEDLINEplus, NLM's consumer health information service, has a general rare diseases page that has been effective in referring the public to the NIH Office of Rare Diseases and was accessed more than 2,600 times last year. In FY 2002, MEDLINEplus added a newsfeed, which includes articles about advances in rare diseases, and an e-mail announcement list, through which the public may obtain information about new topics and links on MEDLINEplus, including the latest information on rare diseases (www.nlm.nih.gov/medlineplus/rarediseases.htm).

ORD staff work closely with NLM staff to incorporate information on clinical trials for rare diseases into the ClinicalTrials.gov database (clinicaltrials.gov).

Online Multiple Congenital Anomaly/ Mental Retardation (MCA/MR) Syndromes is a database of structured descriptions of congenital abnormalities (many of them rare) associated with mental retardation. This database was searched more than 500,000 in 2001 (www.nlm.nih.gov/mesh/jablonski/syndrome_title.html).

Research Support

NLM chairs the Communications Working Group of the Multilateral Initiative on Malaria (MIM), which began in 1997. Its objective is to support African scientists and enable malaria researchers to connect with one another and sources of information through full access to the Internet and the resources of the World Wide Web, as well as create new collaborations and partnerships (www.mimcom.net).

A phase II award will go to the proposal A Multicenter Clinical Trial Using NGI Technology. Next-Generation Internet (NGI) technology will be applied to provide the infrastructure of a multicenter clinical trial of new therapies for adrenoleukodystrophy (ALD), a fatal neurological genetic disorder. This project involves the formation of a worldwide imaging network of clinical institutions to evaluate ALD therapies. The network must provide enough patients for evaluating ALD therapies and can serve as a model for many other disorders. Three centers will collaborate on this project: the Imaging Science and Information Systems center at Georgetown University Medical Center, Kennedy Krieger Institute, and the department of radiology at Johns Hopkins University. NGI technology will be used to speed the transmission and evaluation of high-quality magnetic resonance imaging. Another important feature of this proposal is to gain insight into procedures that ensure medical data privacy and security (www.nlm.nih.gov/research/ngisumphase2.html).

NLM Staff Support

NLM worked with ORD to support the Second Workshop on Trimethylaminuria, held at the Natcher Conference Center in March 2002. A bibliography, reviewed by the Trimethylaminuria Support Group, was distributed to workshop attendees and is available on the NLM Web site. This publication updates one prepared for the First International Workshop on Trimethylaminuria, held at NLM in 1999 (www.nlm.nih.gov/pubs/cbm/trimethylaminuria.html).

Research Initiatives

The National Center for Biotechnology Information (NCBI), a division of NLM, serves as a national public resource for molecular biology information. In this capacity, NCBI establishes and maintains various genomic databases and develops software tools for mining and analyzing these data, all of which are freely disseminated to the biomedical community to facilitate a better understanding of the processes affecting human health and disease.

Human Genetic Map

NCBI is responsible for collecting, managing, and analyzing the growing body of data being generated from the sequencing and mapping initiative of the Human Genome Project. These data are available without restriction to the scientific community and have expedited the decoding of various human chromosomes. By analyzing the DNA sequence of a chromosome, scientists may begin to understand the causes of certain rare diseases. Scientists can determine the organization of the genes on a chromosome, how these genes are expressed, how changes in a gene's DNA sequence gives rise to a disease-causing mutation, and how a chromosome is duplicated and inherited. Scientists have used these strategies to find clues about gene defects on chromosomes 21 and 22 that lead to various rare diseases, including Usher syndrome, DiGeorge syndrome, and Ewing sarcoma—to name a few. NCBI investigators have also played an instrumental role in the identification and analysis of other disease genes and genetic loci, including analysis of genetic data leading to scientific advances in several rare diseases and disorders, such as the identification and analysis of the genes for Kallmann syndrome and neurofibromatosis (NF1). Examples of other rare diseases currently being studied by NCBI investigators are ataxia telangiectasia, hyper-IgE syndrome, and nemaline myopathy.

Genetic Analysis Software

NCBI investigators are working to develop, implement, and disseminate high-performance computational tools and application software packages for the analysis and linkage of genetic data.

FASTLINK is a software program designed by NCBI investigators to conduct genetic linkage analyses—a statistical technique used to study the association of genes and genetic markers that lie near each other on a chromosome. Genes and other genetic markers that are linked tend to be inherited together and therefore can be used to identify and map the location of a particular disease gene. NCBI investigators have used FASTLINK to study hyper-IgE syndrome, a rare immunodeficiency characterized by recurrent skin abscesses, pneumonia, and highly elevated levels of serum IgE. Using FASTLINK, researchers were able to find evidence linking this syndrome to chromosome 4. FASTLINK has been cited in more than 400 published genetic studies, including studies of macular dystrophy, type 1 hereditary sensory neuropathy, and Alström syndrome.

CASPAR (Computerized Affected Sibling Pair Analyzer and Reporter) is a software program designed by NCBI investigators to study the genetics of complex diseases, defined as diseases involving the interaction of multiple genes. It allows a user to explore hypotheses about how different factors may be involved in disease susceptibility. NCBI investigators have used CASPAR for linkage analysis in patients with a form of diabetes.

The PedHunter software was developed to query genealogical databases to determine a connection between a set of relatives that are afflicted with the same disease and to construct a pedigree suitable for input into genetic linkage analysis. NCBI investigators are using PedHunter to query the Amish genealogy database to collect information on various genetic diseases, including nemaline myopathy, which is a rare genetic neuromuscular disorder that is usually apparent at birth and characterized by extreme muscle weakness. Using PedHunter in combination with other genetic analysis software, NCBI investigators have demonstrated that, in the Amish, this disorder is caused by a mutation in the gene for the sarcomeric thin-filament protein slow skeletal muscle troponin T

(TNNT1). TNNT1 maps to chromosome 19 and has been previously sequenced. Further analysis resulted in identification of a stop codon that segregated with the disease. Researchers concluded that Amish nemaline myopathy is a distinct heritable myopathic disorder caused by a *TNNT1* mutation.

The CGH (comparative genomic hybridization) analysis software package is being used by NCBI investigators to model the process of tumor formation in various forms of cancer. The focus of the software is to develop models that relate genetic aberrations with tumor progression. Investigators have used CGH as part of a larger project to search for and identify possible susceptibility loci involved in both breast and bladder cancer. Investigators have also published the results of a case study in which CGH was used to analyze chromosomal abnormalities in a large collection of ovarian cancer samples.

Three-Dimensional Structure Database

NCBI's Structure Research Group maintains a database of experimentally determined three-dimensional biomolecular structures, as well as tools for visualizing and analyzing these structures. Three-dimensional structures offer a wealth of information on biological function of a molecule, mechanisms linked to function, and evolutionary history of and relationships among macromolecules—all valuable clues toward better understanding rare diseases.

For example, in 1995, the structure of leptin—the protein coded for by a gene linked to obesity and forms of diabetes—was predicted by NCBI investigators by use of the structure database. After the discovery of leptin, researchers analyzed the protein's sequence and determined that it exhibited no similarities to other known proteins. NCBI investigators hypothesized that leptin was ancestrally related to at least one other protein whose sequence had diverged such that only a comparison of three-dimensional structures might detect a relationship. Investigators conducted a search of the database to determine whether this protein might adopt a fold pattern or structure similar to that of a protein structure already stored in the database. They discovered that leptin's sequence was compatible with the structure of a family of known proteins and predicted a structural model based on these results. This early prediction was confirmed by cloning the protein's receptor and, more recently, by x-ray structure determination. Now that the structure of leptin has been confirmed, future studies of leptin and other leptin-regulated genes may reveal the mechanisms by which leptin exerts its effect on the body.

Malaria Genetics and Genomics

Malaria is by far the world's most important tropical parasitic disease. The causative agents in humans are four species of a single-celled parasite from the genus *Plasmodium—P. falciparum, P. vivax, P. ovale,* and *P. malariae.* Of these, P. falciparum accounts for most of the infections and is the most lethal. Malaria is a curable disease when promptly diagnosed and adequately treated. Therefore, much research at NIH focuses on the treatment and prevention of malaria.

NCBI, in collaboration with NIAID, supports the efforts to sequence and analyze the complete genome of *P. falciparum,* providing researchers with access to information relative to all of the genes found in this parasite. Genetic investigations of malaria require a genomewide high-resolution linkage map of *P. falciparum.* A collaborative team of NIH investigators, including researchers from NCBI, constructed such a map. The markers, map, and other parameters, are facilitating genome sequence assembly, localization of determinants for such traits as virulence and drug resistance and genetic studies of parasite field populations.

NCBI's Malaria Genetics and Genomics Web page is a resource for data and information relevant to *Plasmodium* in general and, more specifically, to *P. falciparum, P. vivax* (the second most prevalent form of human malaria), and various forms of rodent malaria. From NCBI's site, researchers may access genome maps, linkage markers, and information about genetic studies. Links are also provided for other malaria sites and for genetic data on related para

sites, including NIAID's Malaria Research and Reference Reagent Resource Center (MR4), a central source for quality controlled malaria-related reagents for the international research community. The site also has links to information concerning malaria epidemiology, taxonomy, molecular tools for data analysis, and various malaria research projects being conducted at NIH.

NCBI recently released a new resource for enhancing studies on *Anopheles gambia* (mosquito), the primary vector that transmits human malaria. This information, together with the knowledge gained from the sequences of malaria parasites and the human genome, will provide researchers with a wealth of genomic data necessary for understanding this complex disease and for developing malaria control strategies and improved antimalarial drugs and vaccines.

Additional Human Genome Resources

NBCI makes several other resources available to facilitate the widespread use of human sequence data. The Human Genome Resources Web page is an integrated one-stop genomic information infrastructure for biomedical researchers worldwide. From this Web page, researchers can access the NCBI Map Viewer, which presents a graphic view of available human sequence data, as well as cytogenetic, genetic, physical, and radiation hybrid maps. Researchers may search for a gene or gene marker of interest by querying against the entire human genome or by one chromosome at a time. Query results link to a graphic display of where the gene or gene marker may be viewed in the context of additional data.

NCBI's Genes and Disease Web page is designed to introduce a visitor to the relationship between genetic factors and human disease. It provides information for more than 60 genetic diseases, including numerous rare diseases and disorders. The Online Mendelian Inheritance in Man database (OMIM) is a continuously updated catalog of inherited human disorders and their causal mutations, authored and edited byVictor A. McKusick and colleagues and developed for the Web by NCBI.

One of the many reasons for sequencing the human genome is to gain understanding of the role of a gene, or genes, in human disease. By studying the sequence of a disease gene, whether from humans or other model organisms, researchers can gain important insights into the genetic and environmental basis of disease. The advances outlined here demonstrate the importance and utility of NCBI's computer databases, data analysis tools, and software algorithms in identifying and understanding human disease genes and pave the way for development of novel strategies to diagnose, treat, and ultimately prevent all forms of disease.

Office of Research on Women's Health (ORWH)

Overview

ORWH supported 32 grants that focused on disorders thought to be rare diseases. These disorders include chronic fatigue syndrome, fibromyalgia, rheumatoid arthritis, multiple sclerosis, scleroderma, Sjögren syndrome, systemic lupus erythematosus, gestational diabetes mellitus, temporomandibular joint disease/ temporomandibular disorder (TMJ/TMD), lymphangioleiomyomatosis (LAM), fragile X syndrome (FRX), and osteoarthritis. Because ORWH does not have direct funding authority, these grants were funded through collaborations with 13 NIH Institutes and Centers.

Recent Scientific Advances

Chronic Fatigue Syndrome (CFS)

A total of $5.8 million was spent on CFS research across NIH. These grants were funded by NHLBI, NIDCR, NINDS, NIAID, NIMH, NINR, and NCRR. In FY 2001, ORWH assumed responsibility for coordinating NIH's CFS research efforts through the trans-NIH CFS Working Group (CFSWG). The trans-NIH membership includes ORWH, the Office of Behavioral and Social Sciences Research (OBSSR), the Office of Dietary Supplements, NIAID, NIAMS, the Center for Scientific Review, NCCAM, NCRR, NIAAA, NICHD, NIDCR, NIMH, NIEHS, NINDS, NINR, and NHLBI. During this period, ORWH dedicated a portion of its Web site to CFS and participated with the CFIDS (chronic fatigue and immune dysfunction syndrome) Association of America in planning scientific conferences focused on specific aspects of the disease to stimulate and improve the quality of research in this area.

Scleroderma

ORWH cofunded a grant with NCCAM that focused exclusively on scleroderma and supported several other grants that included this disorder and other autoimmune diseases. Two basic science grants funded with NIAMS focused on evaluating the effectiveness of treating primary fibroblast cultures from the lungs of scleroderma patients with curcumin. Investigators found that this treatment inhibits collagen accumulation and promotes cell death in these cultures while having no effect on normal lung fibroblasts. The second grant evaluated the significance of autoantibodies in these patients.

Fibromyalgia

This project, cofunded by ORWH and NIAMS, focuses on improving clinical treatment of fibromyalgia by increasing understanding of the effectiveness of new drug regimens and factors affecting patient responses to these treatments. Investigators have developed an effectiveness research method that uses patient-focused (N-of-1) trials, then combines the results to obtain population estimates of treatment effectiveness and make more informed decisions regarding a patient's treatment.

Rheumatoid Arthritis (RA)

Along with the four Autoimmune Centers of Excellence grants that ORWH cofunds with NIAID, ORWH and NIAMS supported two RA-specific grants: a clinical research grant and a longitudinal registry of African American women diagnosed with early RA. The clinical research study focuses on a potential target for chondroprotective therapy based on the finding that RA leads to synovial hyperplasia with local invasion of bone and cartilage. ORWH funded the lead research group that is establishing a multi-institutional longitudinal registry of African American women diagnosed with early RA to identify genetic and nongenetic prognostic factors of disease outcome to permit prospective analyses of factors predictive of the clinical phenotype and outcomes.

Multiple Sclerosis (MS)

These grants, funded by ORWH, NIAID, NIAMS, and NEI, support clinical trials that focus on identifying subsets of MS patients, permitting evaluation of treatment outcomes

within the various subsets. Investigators categorized MS patients based on whether they have clearly defined relapses, have relapsing-remitting MS, or are progressing. Progressing patients are further categorized based on whether they initially experienced relapses or whether they had deteriorated slowly without evidence of relapses or remissions. The NEI grant focuses on defining the visual profile of MS in a large cohort of 400 patients and determining which measures best identify visual dysfunction in MS patients. The investigators will then determine the relationship of visual function and disease-specific health-related quality of life in patients with MS.

Systemic Lupus Erythematosus (SLE)

ORWH has cofunded SLE grants with NIAID, NIAMS, and NIEHS. Four grants explore genetic aspects of SLE, including two gene-mapping grants and two clinical therapy grants. Four Autoimmune Centers of Excellence grants, cofunded by ORWH and NIAID, support large translational studies that bridge basic science with clinical research. Within these large center grants, SLE-specific studies are being conducted with various other studies focused on autoimmune disorders such as RA and MS.

Sjögren Syndrome (SS)

SS is a common rheumatic autoimmune disease that initially affects the salivary and lacrimal glands but can affect the lungs, kidneys, central nervous system, and vasculature. The etiology and pathological mechanisms are unknown for this disease, and therapy is available but far from ideal. SS is an autoimmune disease, as evidenced by the almost universal presence of autoantibodies in the sera of patients. Most patients have antibodies binding one or more components of the Ro/La (or SSA/SSB) ribonucleoprotein particle, which is found in every mammalian cell type examined and whose function is not completely known. There are several animal models of SS, but none have high levels of anti-Ro or anti-La. Thus, although these models may prove useful for studying some aspects of the disease, insight into the origin and pathogenic potential of autoimmunity targeting the Ro ribonucleoprotein cannot be sought or found.

Osteoarthritis

This translational research, supported by both ORWH and NIAMS, is aimed at developing new therapies for elderly, osteoarthritic women who currently have only nonsteroidal anti-inflammatory drugs (NSAIDs) to alleviate their symptoms. Unfortunately, this age group is at greatest risk of developing serious side effects from NSAIDs. Building on basic research findings, researchers have shown that prophylactic oral administration of doxycycline markedly reduces the severity of cartilage damage in a canine model of osteoarthritis. Beneficial protective effects were apparent, even when therapy was initiated after cartilage lesions were established. Similar results have been noted in other animal models of osteoarthritis, such as guinea pigs and rabbits. Based on the encouraging basic research findings, a randomized placebo-controlled clinical trial will examine the effects of doxycycline and its ability to prevent the progression of early knee osteoarthritis in elderly women.

Diabetes Mellitus and Gestational Diabetes Mellitus (GDM)

Working with NIDDK, ORWH supported five grants focused on diabetes mellitus and/or GDM. ORWH, with NIDDK, funded a large multicenter clinical trial designed to determine whether type 2 diabetes can be prevented or delayed in a population of high-risk individuals, of which 50% represent minority populations. Of the total number of participants, 13% are women with a history of GDM.

TMJ/TMD

ORWH is cofunding two pain-control grants with NIDCR. The behavioral grant is to test the efficacy of psychological interventions, pharmacological interventions, and a combination of the two to reduce pain and improve function in individuals with TMJ. The second grant seeks to elucidate the cellular mechanisms of paclitaxel-induced painful peripheral neuropathy in the rat. By improving understanding of the cellular

mechanisms of neuropathic pain, these studies can potentially provide insight into the pathophysiology and treatment of orofacial neuropathies. Paclitaxel (Taxol) is widely used for the treatment of many different types of carcinomas. Currently, the dose of paclitaxel that can be tolerated by patients is limited primarily by the development of a painful peripheral neuropathy characterized by parenthesis, myalgia, and arthralgia.

Fragile X Mental Retardation (FRX) Gene Premutation

FRX is a form of congenital mental retardation in humans, usually resulting from lack of expression of the fragile X mental retardation gene *(FMR1)*. Unaffected carriers, or so-called FRX premutation carriers, show an increased prevalence of premature ovarian failure (POF), estimated to affect 1% of women worldwide. The prevalence of POF in FRX premutation carriers has been reported to be 16%. The study will characterize the cell-specific *FMR1* gene expression changes in normal human and mouse ovaries through their respective reproductive cycles and define the physiology of hypothalamic-pituitary-ovarian function in human female FRX premutation carriers. A repository of genetic material and extensive phenotypic information about women with POF will be established that could eventually be used to test other candidate genes for POF.

LAM

Through NHLBI, ORWH is supporting the LAM patient registry through a consortium of six clinical centers and referring physicians who treat LAM patients. The cohort of identified individuals with LAM will be used to characterize the clinical features of subjects and provide information on the natural course of the disease. The registry will include clinical data and tissue samples that will be used to study the course of the disease and assess interventions. Data and tissue samples will also be banked for future studies.

Vulvodynia

ORWH cofunded two grants with NICHD to elucidate the pathophysiological mechanisms of vulvodynia to develop improved treatment strategies and to assess the differences in specific neuroimmunological characteristics between women with vulvodynia and asymptomatic control subjects. Results from this study will lead to improved understanding of neuroimmunological alterations in women with vulvodynia that will direct future therapeutic strategies for this disorder.

New and Planned Extramural Research Initiatives

Efforts in FY 2002 are concentrated on a new program announcement (PA) to stimulate and broaden the scope of CFS research. This PA will be based on the recommendations of an ORWH-sponsored state-of-the-science conference held in October 2000. Future efforts will also focus on developing a research plan and a scientific symposium to stimulate interdisciplinary study of CFS.

ORWH will join with NIAMS and NIA in the public-private Osteoarthritis Initiative.

RFPs and RFAs for FY 2001

- Hyperaccelerated Award/Mechanisms in Immune Disease Trials—NIAID/NIA/NIAMS/NIDDK/NINDS/ORWH
- Environment/Infection/Gene Interactions in Autoimmune Diseases—NIEHS/NIAID/NIDDK/NIDCR/ORWH
- Autoimmunity Centers of Excellence—NIAID/NIDDK/NIAMS/ORWH
- Basic and Clinical Research on Fibromyalgia—ORWH/NIAMS/NIDR/NINDS/OAM/OBSSR
- Sex-Based Differences in the Immune Response—ORWH/NIAID/NINDS/NIAMS/National MS Society

Workshops, Symposia, and Meetings

Chronic Fatigue Syndrome (CFS) Conference, NIAID

This conference was divided into seven topic areas: neuroendocrinology, cognition, chronic pain, sleep, immunology, orthostatic intolerance/neurally mediated hypotension, and fatigue, functional status, and disability. The goals for this meeting were to focus on CFS research areas in which information is both most advanced and exciting, summarize what is known and identify important gaps in knowledge, garner perspectives from expert investigators not currently working on the CFS problem, and identify expert investigators who might be attracted to study CFS as a clinical problem (www4.od.nih.gov/orwh/state-of-science.pdf).

FASEB Research Conference on Autoimmunity, NIAID

This meeting focused on the latest developments in the field of autoimmunity, especially how recent advances in basic immunology and cell biology have influenced this field. The conference presented a comprehensive view of basic immunological mechanisms related to autoimmunity, including those involved in the autoimmune process and immune intervention.

Health Disparities in Arthritis and Musculoskeletal and Skin Diseases, NIAMS

The purpose of this conference was to highlight current knowledge on genetic and environmental factors that play a role in the marked differences in prevalence, morbidity, and disability associated with specific rheumatic, musculoskeletal, and skin conditions in various populations; identify challenges and emerging opportunities for research in these areas; and highlight intervention strategies that could provide models for reducing these disparities.

Treatment of Salivary Gland Disorders: Alternative Approaches, NIDCR

This conference brought together scientists with an interest in basic and clinical research on the treatment of salivary gland dysfunction with alternative approaches to foster research collaborations and future directions.

Meetings and Workshops

Several ORWH staff, including its director, Vivian W. Pinn, serve on various steering/coordinating committees and working groups for several rare diseases covered in this report. For example, the director is a member of the Autoimmune Diseases Coordinating Committee, which was very active during FY 2001. Lisa Begg, ORWH Director of Research Programs, served on the steering committee of the Autoimmune Diseases Coordinating Committee Working Groups, which prepared the Autoimmune Diseases Research Plan. Eleanor Hanna, on behalf of ORWH, chairs the trans-NIH Working Group on CFS.

Office of Rare Diseases (ORD), Office of the Director

Overview

The main objective of ORD is to stimulate and coordinate research on the more than 6,000 rare diseases known today. *Rare disease* is defined as a disease, condition, disorder, or syndrome for which there are fewer than 200,000 affected people in the United States.

Research Initiatives

Highlights of ORD Activities

As part of its ongoing support of rare diseases initiatives, ORD

- Completed the *Report on Research on Rare Diseases in Children: FY 2000 to FY 2005*. Section 2801 of Public Law 106-310, the Children's Health Act, required this one-time report on the contributions and research advances and activities of the NIH institutes and centers (ICs) for FY 2000 and research plans for FYs 2000–2005.
- Jointly with the ICs, other Federal agencies, voluntary health organizations, and the pharmaceutical and biotechnological industries, prepared the report *Analysis by and Recommendations of the Special Emphasis Panel of the National Institutes of Health on the Coordination of Rare Diseases Research*, submitted by NIH, on steps to coordinate rare disease research programs.
- Developed a request for proposal with NHGRI to establish a Genetic and Rare Diseases Information Center to answer questions from the general public, including patients and their families, health-care professionals, and biomedical researchers. The Information Center is now operational, following the award of a contract to Aspen Systems Corporation see below (for contact information).
- Developed and supports the Medical Genetics and Rare Disorders subfile of the Combined Health Information Database (CHID), providing information about and available from voluntary patient support groups. Information on approximately 1,500 voluntary patient support groups is available through the ORD and CHID Web sites.
- Responded to requests for information on highly technical matters and public policy on and public interest in research on rare diseases.
- Prepared the NIH Director's annual report to Congress on rare diseases research activities sponsored by NIH. All reports are published on ORD's Web site at rare diseases.info.nih.gov.
- Initiated discussion of an extramural program of research grants and centers focusing on rare diseases and an intramural program at NIH to supplement existing rare diseases research activities. Both programs were initiated in FY 2002 and will be fully implemented in FY 2003.

Scientific Workshops

In FY 2001, ORD cosponsored 54 scientific workshops with NIH research ICs. A list of the FY 2001 ORD-cosponsored workshops appeared in last year's annual report. Workshops on rare diseases research are funded if a particular scientific opportunity exists or if very little (if any) research is under way. Findings from an evaluation of the workshops show that the workshops are an effective means of generating novel research ideas and grant applications in rare diseases areas that otherwise might not attract much attention.

In FY 2002, ORD provided support to cosponsoring the following 58 workshops with the primary sponsoring NIH institute(s).

NIAAA

- Mechanisms of Alcoholic Pancreatitis

NIAID

- Development of Small Animal Models for Bacterial Pathogens With Potential Use as Bioterrorism Agents
- Hepatic Inflammation and Immunity
- Polyomavirus Nephropathy in Immunosuppressed Kidney and Other Solid Organ Transplant Recipients
- Scientific Workshop on the Role of the NADPH Oxidase in the Regulation of Inflammation
- Drugs Against Tropical Protozoan Parasites
- Lymphatic Continuum
- Autoantibodies as Predictors of Disease

NIAMS

- Multiple Hereditary Exostoses

NCI

- Fourth Tri-National Meeting on Collaborative Chornobyl Thyroid Research Projects
- Estimation of Thyroid Doses Resulting From Atmospheric Nuclear Weapons Tests: Comparison of American and Russian Experience
- Studies of the Etiology of Brain Cancer—International Workshop
- U.S.-Ukrainian Chornobyl Collaborators Leukemia Meeting
- Lymphomatoid Granulomatosis: Pathogenesis, Pathobiology, and Treatment
- Molecular Pathogenesis of Human Hepatocellular Carcinoma—International Meeting
- Nutritional Genomics and Proteomics in Cancer Prevention
- Pediatric Long-Term Followup Clinic and Program Workshop

NICHD

- Inborn Errors of Cholesterol Synthesis
- Rare Causes of Primary and Secondary Ovarian Insufficiency in Adolescents and Young Women
- Pediatric Critical Care
- Chronic Pelvic Pain: Pathogenic Mechanisms, Treatment Innovations, and Research Implications
- Research Planning Workshop on Intersex Research
- International Workshop on Maternal Phenylketonuria

NICHD/NCMRR

- Brain Computer Interfaces (BCI) for Communication and Control

NIDCR

- Sixth Research Workshop on Head and Neck Cancer
- Quality of Life Conference on Head and Neck Cancer

NIDDK

- Genetic Modifiers of Mendelian Diseases
- Asymptomatic Primary Hyperparathyroidism: A Perspective for the 21st Century
- Innovative Therapeutic Approaches to Inborn Errors of Metabolism

NIEHS

- Thyroid Hormones, Brain Development and the Environment
- International Epidemiology Association/
- World Congress of Epidemiology
- Information Workshop on Alpha-1 Antitrypsin Deficiency

- Development of Outcome Measures and Consensus on Design Issues for Myositis Clinical Trials—2nd Meeting
- Symposium on the National Children's Study
- Cellular and Molecular Biology of Xenobiotic Transport at the Blood-Brain Barrier
- Built Environment—Healthy Communities, Healthy Homes, Healthy People: Multilevel, Interdisciplinary Research Approaches

FIC

- Zoonotic Infections in the US and Abroad: Research Needs and Opportunities

NHLBI

- Translational Research in Primary Pulmonary Hypertension

NHGRI

- Clinical Research Workshop on Lowe Syndrome
- Exploration of Therapeutic Interventions for Congenital Disorders of Glycosylation—Type 1A
- 2nd NIH Conference on Holoprosencephaly and Early Embryonic Development

NIMH

- Psychosocial Treatment Research in Children With Autism and Other Pervasive Developmental Disorders
- Neurobiology of Bipolar Disorder
- Suicide Risk and Physical Illness
- Asian/Pacific Islanders Living With AIDS: Special Issues and Future Research Directions
- Mental Health Research Issues in HIV and Aging
- Mechanisms of Neuropathogenesis of HIV-Associated Progressive Multifocal Leukoencephalopathy (PML): Implications for Therapy
- Challenges of Treating Anorexia Nervosa

NINDS

- Mucopolysaccharidosis: The Therapeutic Strategies for the Central Nervous System
- Research and Clinical Practice in Joubert Syndrome
- International Symposium on Pediatric Neurotransmitter Disease
- Workshop on Brain Banking
- Familial Dysautonomia
- Tuberous Sclerosis Complex (TSC) Research Conference
- Childhood Motor Disorders
- Barth Syndrome

NINR

- Ethical Challenges of End-of-Life Research That Involves Persons With Genetic Disorders
- Increasing Nursing Research Opportunities in Biodefense

In addition, ORD cosponsored with the Office of Orphan Products Development, Food and Drug Administration, the workshop Therapies for Rare Diseases: From Bench to Marketplace.

Genetic and Rare Diseases Information Center

Established by NHGRI and ORD, the Genetic and Rare Diseases Information Center employs experienced information specialists to answer questions from the general public, including patients and their families, health-care professionals, and biomedical researchers. Information from the Center includes

- Publicly available and reliable information on the disease or condition.
- Locations of genetic counseling centers that are available for consultation.

- Summaries and locations of current and planned research related to rare diseases and genetic disorders.
- Names, locations, and types of major printed or audiovisual material available through voluntary patient support groups.
- Disease-specific fact sheets.
- Related Web links.

The Information Center does not provide medical advice or treatment, nor does it diagnose illness.

The Genetic and Rare Diseases Information Center operates Monday through Friday, Noon–6 p.m. eastern time. The Information Center can be reached by calling 888–205–2311 (TTY 888–205–3223); by faxing 240–632–9164; or by e-mailing gardinfo@nih.gov. The Information Center can also be reached by U.S. Mail sent to The Genetic and Rare Diseases Information Center, P.O. Box 8126, Gaithersburg, MD 20898–8126. Responses to all inquiries will be made within 5–7 business days.

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Acronyms

α-1	α_1-antitrypsin deficiency
AA	aplastic anemia
AARP	American Association of Retired Persons
AAT	α1-antitrypsin
AAV	adeno-associated virus
ACE	Autoimmunity Center of Excellence
ACRIN	American College of Radiology Imaging Network
ADHD	attention deficit hyperactivity disorder
AGS	Alagille syndrome
AIDS	acquired immune deficiency syndrome
ALD	adrenoleukodystrophy (NLM)
ALD	alcoholic liver disease (NIAAA)
ALL	acute lymphoblastic leukemia
ALPS	autoimmune lymphoproliferative syndrome
ALS	amyotrophic lateral sclerosis
ALTS	ASCUS/LSIL Triage Study
AN	anorexia nervosa
ANS	autonomic nervous system
ApoB	apoprotein B
APS	antiphospholipid syndrome
ARDS	acute respiratory distress syndrome
ARVD	arrhythmogenic right-ventricular dysplasia
ART	antiretroviral therapy
ASCUS	atypical squamous cells of undetermined significance
ASL	airway surface liquid
AT	ataxia telangiectasia
ATM	ataxia telangiectasia mutated
AZT	azidothymidine
BAA	broad agency announcement
BAMSG	Bacteriology and Mycology Study Group
BDD	body dysmorphic disorder
BH4	tetrahydrobiopterin
BHD	Birt Hogg Dube syndrome
BL	Burkitt lymphoma
BN	bulimia nervosa
BPD	bronchopulmonary dysplasia
BPES	blepharophimosis–ptosis–epicanthus inversus syndrome
BS	Bloom syndrome
BSE	bovine spongiform encephalopathy
BTK	Bruton's tyrosine kinase
CACP	camptodactyly–arthropathy–coxa vara–pericarditis
CAH	congenital adrenal hyperplasia
CAM	complementary and alternative medicine
CASG	Collaborative Antiviral Study Group
CASPAR	Computerized Affected Sibling Pair Analyzer and Reporter
CC	Warren Grant Magnuson Clinical Center
CCHS	congenital central hypoventilation syndrome
CDC	Centers for Disease Control and Prevention
CDG	congenital disorders of glycosylation
CDH	congenital diaphragmatic hernia
CF	cystic fibrosis
CFS	chronic fatigue syndrome
CGD	chronic granulomatous disease
CGH	comparative genomic hybridization
CHD	coronary heart disease
CHID	Combined Health Information Database
CJD	Creutzfeldt-Jakob disease
CL/P	cleft lip and cleft palate
CMV	congenital cytomegalovirus
CNS	central nervous system
COPD	chronic obstructive pulmonary disease
CRADA	Cooperative Research and Development Agreement
CRF	corticotropin-releasing factor
CS	Cockayne syndrome
CT	computed tomography
CYP2E1	cytochrome P450 2E1
DA	dopamine
DbpA	decorin-binding protein A
DDG	Drug Development Group
DES	diethylstilbestrol
DKC	dyskeratosis congenita
DM	myotonic dystrophy
DMD	Duchenne muscular dystrophy
DNA	deoxyribonucleic acid
DS	Down syndrome
DTR&D	Division of Treatment Research and Development
EBV	Epstein-Barr virus
ECT	electroconvulsive therapy
EDA	ectodermal dysplasia (anhidrotic)
EDS	Ehlers-Danlos syndrome
EFT	Ewing family tumor
ELST	endolymphatic sac tumor
EOP	early-onset periodontitis
ESRD	end-stage renal disease
FA	Fanconi anemia
FAEE	fatty acid ethyl ester
FAS	fetal alcohol syndrome
FBN1	fibrillin 1
FDA	Food and Drug Administration
FDNIB	familial dementia with neuroserpin inclusion bodies
FGFR3	fibroblast growth factor receptor type 3
FH	familial hypercholesterolemia
FHBL	familial hypobetalipoproteinemia
FHCM	familial hypertrophic cardiomyopathy

FMF	familial Mediterranean fever
FRDA	Friedreich ataxia
FRX	fragile X syndrome
FSHD	facioscapulohumeral muscular dystrophy
GAS	group A streptococcus
GBS	group B streptococcus
GCPS	Greig cephalopolysyndactyly syndrome
GDM	gestational diabetes mellitus
GFAP	glial fibrillary acidic protein
GIST	gastrointestinal stromal tumor
GM-CSF	granulocyte-macrophage colony-stimulating factor
GVHD	graft versus host disease
HbF	fetal hemoglobin
HCT	hematopoietic cell transplantation
HCV	hepatitis C virus
HD	Huntington disease
HDL	high-density lipoprotein
HDN	hemolytic disease of the newborn
HED	hypohidrotic ectodermal dysplasia
HEV	hepatitis E virus
HGF	hereditary gingival fibromatosis
HGP	Human Genome Project
HGPS	Hutchinson-Gilford progeria syndrome
HHV	human herpesvirus
HIV	human immunodeficiency virus
HPT-JT	hyperparathyroidism-jaw tumor (syndrome)
HPV	human papillomavirus
HRV	heart rate variability
HSC	hepatic stellate cell (NIAAA)
HSC	hematopoietic stem cells (NIAID)
HSS	Hallervorden-Spatz syndrome
HSV	herpes simplex virus
IBS	irritable bowel syndrome
ICAM-1	intercellular adhesion molecules-1
ICs	institutes and centers
IDF	Immune Deficiency Foundation
IFN	interferon
IGF	insulinlike growth factor
IgG	immunoglobulin G
IgM	immunoglobulin M
IGR	interferon-γ receptor
IIM	idiopathic inflammatory myopathy
IKBKAP	IκB kinase complex-associated protein
IL	interleukin
INCL	infantile neuronal ceroid lipofuscinosis
IND	investigational new drug
IP	incontinentia pigmenti
IP2	familial incontinentia pigmenti
IPF	idiopathic pulmonary fibrosis
ITP	immune thrombocytopenic purpura
JMF	Jeffrey Modell Foundation
JRA	juvenile rheumatoid arthritis
KS	Kaposi sarcoma
KSHV	Kaposi sarcoma herpesvirus
KTWS	Klippel-Trenaunay-Weber syndrome
LAM	lymphangioleiomyomatosis
LBP	lipopolysaccharide-binding protein
LCA	Leber's congenital amaurosis
LD	lymphedema
LDL	low-density lipoprotein
LF	Li-Fraumeni syndrome
LGV	lymphogranuloma venereum
LJP	localized juvenile periodontitis
LPS	lipopolysaccharide
L-R	left-right
LQTS	long QT syndrome
LSIL	low-grade squamous intraepithelial lesion
LVAS	large vestibular aqueduct syndrome
MAS	McCune-Albright syndrome
MD	myelodysplastic syndrome
MDD	Medications Development Division
MDMA	methylenedioxymethamphetamine
MDP	medications development program
MEN1	multiple endocrine neoplasia type 1
MHC	major histocompatibility complex
MP	mannoprotein
MRI	magnetic resonance imaging
MS	multiple sclerosis
MSC	mesenchymal stem cell
MSH	Multicenter Study of Hydroxyurea
MTP	microsomal triglyceride transfer protein
NBS	Nijmegen breakage syndrome
NCCAM	National Center for Complementary and Alternative Medicine
NCBI	National Center for Biotechnology Information
NCI	National Cancer Institute
NCMHD	National Center on Minority Health and Health Disparities
NCRR	National Center for Research Resources
NCS	National Children's Study
NDA	new drug application
NEC	necrotizing enterocholitis
NEI	National Eye Institute
NER	nucleotide excision repair
NF1	neurofibromatosis type 1
NF2	neurofibromatosis type 2
NGI	Next-Generation Internet
NHGRI	National Human Genome Research Research Institute
NHL	non-Hodgkin lymphoma
NHLBI	National Heart, Lung, and Blood Institute
NIA	National Institute on Aging
NIAAA	National Institute on Alcohol Abuse and Alcoholism
NIAID	National Institute of Allergy and Infectious Diseases
NIAMS	National Institute of Arthritis and Musculoskeletal and Skin Diseases

NICHD	National Institute of Child Health and Human Development
NIDA	National Institute on Drug Abuse
NIDCD	National Institute of Deafness and Other Communication Disorders
NIDCR	National Institute of Dental and Craniofacial Research
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NIMH	National Institute of Mental Health
NINDS	National Institute of Neurological Disorders and Stroke
NINR	National Institute of Nursing Research
NLM	National Library of Medicine
NO	nitric oxide
NOS	nitric oxide synthase
NPC	Niemann-Pick type C disease
NSAIDs	nonsteroidal anti-inflammatory drugs
OA1	ocular albinism type 1
OAM	Office of Alternative Medicine (now NCCAM)
OBSSR	Office of Behavioral and Social Sciences Research
OCA	oculocutaneous albinism
ODS	Office of Dietary Supplements
ORD	Office of Rare Diseases
ORWH	Office of Research on Women's Health
PA	program announcement
PBD	peroxisomal biogenesis disorder
PC	phosphorylcholine
PCD	primary ciliary dyskinesia
PCP	phencyclidine
PCT	porphyria cutanea tarda
PCV	procarbazine, CCNU (lomustine), and vincristine
PD	Parkinson disease
PDT	photodynamic therapy
PEDI	Pediatric Evaluation of Disabilities Inventory
PEL	primary effusion lymphoma
PEO	progressive external ophthalmoplegia
PHP	pseudohypoparathyroidism
PNH	paroxysmal nocturnal hemoglobinuria
POF	premature ovarian failure
PPH	primary pulmonary hypertension
PPHN	persistent pulmonary hypertension of the newborn
PTH	parathyroid hormone
PWS	Prader-Willi syndrome
PXE	pseudoxanthoma elasticum
RA	rheumatoid arthritis
RAID	Rapid Access to Intervention Development
RAPID	Rapid Access to Prevention Intervention Development
RBC	red blood cell
RCC	renal cell carcinoma
RFA	request for applications
RFP	request for proposals
RMD	Rehabilitation Medicine Department
RNA	ribonucleic acid
RPE65	retinal pigment epithelium 65-kDa protein
rTMS	repetitive transcranial magnetic stimulation
RTS	Rothmund-Thompson syndrome
SADDAN	severe achondroplasia with developmental delay and acanthosis nigricans
SAMe	*S*-adenosylmethionine
SAMHSA	Substance Abuse and Mental Health Services Administration
SBIR	Small Business Innovative Research
SCD	sickle cell disease
SCID	severe combined immunodeficiency
SCOR	Specialized Center of Research
SCPB	streptococcal C5a peptidase
SCT	stem cell transplantation
SES	socioeconomic status
SIDS	sudden infant death syndrome
SLE	systemic lupus erythematosus
SLOS	Smith-Lemli-Opitz syndrome
SMS	Smith-Magenis syndrome
SNP	single nucleotide polymorphism
SPIRCAP	Strategic Program for Innovative Research on Cocaine Addiction Pharmacotherapy
SPS	stiff person syndrome
SS	Sjögren syndrome
STD	sexually transmitted disease
TCDD	tetrachlorodibenzo-*p*-dioxin
TDII	thanatophoric dysplasia type II
TH	thyroid hormone
TLR4	toll-like receptors
TMD	temporomandibular disorder
TMJ	temporomandibular joint disease
TNFα	tumor necrosis factor α
TPO	thrombopoietin
TRAIL	TNF-related apoptosis-inducing ligand
TSC	tuberous sclerosis complex
TSE	transmissible spongiform encephalopathy
TTP	thrombotic thrombocytopenic purpura
URO-D	uroporphyrinogen decarboxylase
UV	ultraviolet
VA	Department of Veterans Affairs
VCFS	velocardiofacial syndrome
VHL	von Hippel-Lindau
VLDL	very low density lipoprotein
VWD	von Willebrand disease
VWF	von Willebrand factor
WG	Wegener's granulomatosis

WHO	World Health Organization
WMS	Williams syndrome
WNV	West Nile virus
WS	Waardenburg syndrome (NIDCD)
WS	Werner syndrome (NIA)
XHIM	X-linked hyper-IgM syndrome
XLA	X-linked agammaglobulinemia
XLP	X-linked lymphoproliferative disease
XP	xeroderma pigmentosum

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